The effect of inflammation and damage to lymph node structures on durable protective immunity following yellow fever vaccination

Protocol Number: JC3318

National Clinical Trial (NCT) Identified Number: NCT04269265

Principal Investigator: Timothy Schacker, MD

Funded by: National Institute of Allergy and Infectious Diseases

Version Number: 2.0

14 August 2023

Summary of Changes from Previous Version:

Version	Affected Section(s)	Summary of Revisions Made	Rationale
1.2	**1.1, 2.1, 8.2, 10.1.5**	**Clarified endpoints, corrected inclusion criteria, corrected PI contact phone number**	**Clarification and correction**
1.3	**1.1, 1.2, 1.3, 2.1, 2.2, 2.3.1, 4.1, 5.1, 8.2, 8.3.3.1, 10.1.10, 10.2**	**Updated vaccine information to Stamaril, added pregnancy test to indicated visits, removed all mention of colon and rectal biopsies, improved formatting for ease of reading, corrected inclusion criteria, updated AE grading scale, clarified reporting requirements, added key roles, addition of rectal swabs**	**Stamaril is the available yellow fever vaccine, correction and update of visit procedures, overall effort to make protocol consistent, up to date, and clear**
1.4	**1.3, 2.3.3., 7.2, 8.3.4, 8.3.6, 10.1.4, 10.1.9.1, 10.1.9.2**	**Added Joshua Rhein at UMN as a Co-I; Medical history will be taken at screening and not baseline; Addressed comments from Ugandan Scientific Council Review: update title to include "yellow fever", update assessment of risk and benefits, JCRC has insurance to cover participants with study procedure complications, samples will be destroyed if a participant is discontinued from the study, all SAEs in Minnesota will also be reported to the Ugandan IRB, materials will not be stored for future research, additional information on data management, records will be kept at JCRC for a minimum of 5 years**	**This version reflects revisions to the protocol as requested by the Ugandan Scientific Council Review.**
1.5	**1.2, 1.3, 2.3.1, 5.1, 5.2, 5.3, 6.2.1, 7.2, 8.2, 8.3.6, 8.4.2, 10.1.8.1**	**Added AE assessment and COVID-19 screenings; modified the schedule of stool collection and rectal swab for the microbiome study; corrected the schema to match the schedule of activities; moved all Day 1 procedures expect the yfv to baseline; changed I/E criteria from >18 years to ≥18 years; added pregnancy within the first 28 days as an exclusion criteria; added pregnancy tests to baseline and month 18 (prior to leukapheresis); added Fairview Uptown and Park Nicollet Travel Clinics as vaccine administration sites; updated the reporting and**	**Adding changes to match responses to JCRC IRB application as well as to specify the Minnesota details.**

		storage to match the JCRC IRB application; removed "AHC services" for data management	
1.6	**1.2, 1.3, 2.3.1, 8.2, 10.1.5**	**Added NCT to cover page; Removed rectal swabs and stool collection at day 12 and week 3; Added stool collection at day 10 and week 4 and included storage of adipose tissue; Updated the safety oversight from DSMB to two ISM – one for each site; Added YF-VAX to the list of yf vaccines as it is available again; added JCRC investigator names per IRB comments**	
1.7	**1.2, 1.3, 8.2**	**Changed the screening period to Day -42 to 1 and the baseline period to Day -35 to 1; Large volume blood draw (60 cc) will be done if a participant is not eligible for leukapheresis; corrections to section 8.2 regarding stool collection to match the schedule of activities**	
1.8	**1.2, 1.3, 4.1, 8.2,**	**Moved 2nd leukapheresis from month 18 to month 6 and extended the month 6 timepoint**	
1.9	**1.2, 1.3, 8.2, 10.1.6,**	**Per Ugandan National Council, the JCRC ICFs will be reviewed on site by the JCRC internal monitor; remove collection of adipose tissues (baseline and week 3)**	
2.0	**10.1.8.2**	**We have added the following to "section 10.1.8.2 Study records retention" of the protocol: "HIPAA authorizations are to be retained for 6 years from the date of its creation or the date when it was last in effect, whichever is later."**	**Modification submitted after QA Human Research Audit.**

Key Roles and Contact Information

Timothy Schacker, M.D., Principal Investigator
University of Minnesota
420 Delaware Street SE
Minneapolis, MN 55455
612-624-9955
Schac008@umn.edu

Cissy Kityo, M.D., Ph.D., Co-Investigator
Joint Clinical Research Centre
Plot 101 Lubowa Estates, off Entebbe Road
P.O. Box 10005
Kampala, Uganda
ckityo@jcrc.org.ug

Daniel Douek, M.D., Ph.D., Collaborator
National Institute of Allergy and Infectious Disease (NIAID)
Vaccine Research Center
Human Immunology Section
Building 40, Room 3504
MSC 3022
Bethesda, MD 20898
ddouek@mail.nih.gov

Cavan Reilly, Ph.D., Biostatistician
University of Minnesota
2221 University Avenue SE
Minneapolis, MN 55414
612-624-9644
cavanr@biostat.umn.edu

Erika Helgeson, Ph.D., Biostatistician
University of Minnesota
2221 University Avenue SE
Minneapolis, MN 55414
612-626-1166
helge@umn.edu

Joshua Rhein, M.D., Co-Investigator
University of Minnesota
420 Delaware St SE
Minneapolis, MN 55455
612-624-9996
rhei0005@umn.edu

Henry Mugerwa, M.D., Ph.D., Co-Investigator

Joint Clinical Research Centre
Plot 101 Lubowa Estates, off Entebbe Road
P.O. Box 10005
Kampala, Uganda
hmugerwa@jcrc.org.ug

Francis Ssali, M.D., Co-PI, DED
Joint Clinical Research Centre
Plot 101 Lubowa Estates, off Entebbe Road
P.O. Box 10005
Kampala, Uganda

Stella Namuddu, M.D., Co-Investigator
Joint Clinical Research Centre
Plot 101 Lubowa Estates, off Entebbe Road
P.O. Box 10005
Kampala, Uganda

Mary Nakibuuka, M.D., Co-Investigator
Joint Clinical Research Centre
Plot 101 Lubowa Estates, off Entebbe Road
P.O. Box 10005
Kampala, Uganda

Table of Contents

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

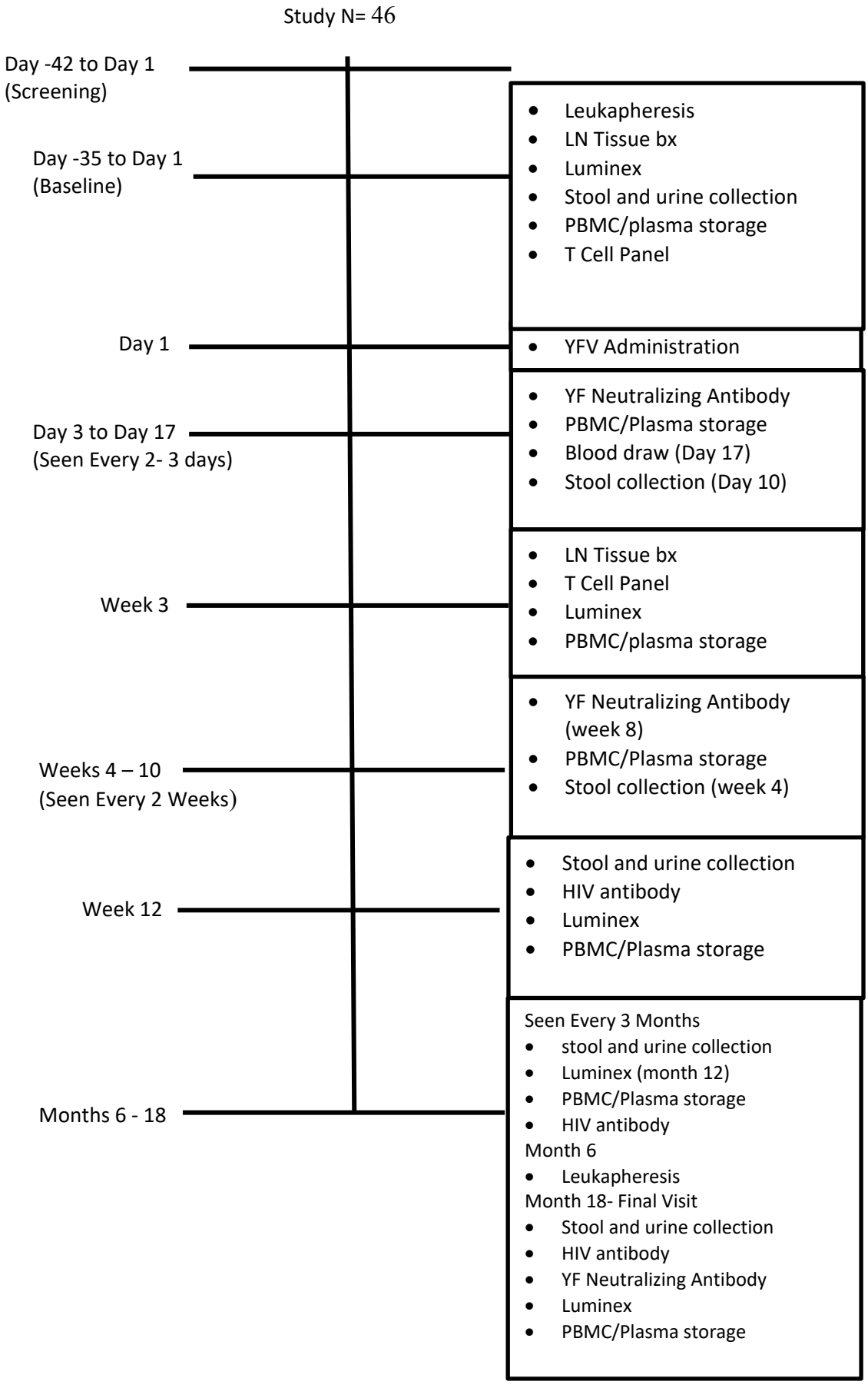
1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	The effect of inflammation and damage to lymph node structures on durable protective immunity following yellow fever vaccination
Study Description:	Our hypothesis is that infections other than HIV can cause lymph node (LN) inflammation and collagen damage to the fibroblastic reticular cell network (FRCn), which will lead to CD4 T cell depletion and impaired vaccine responses. This protocol will study yellow fever vaccine (YFV) in two cohorts of people, one from Uganda and the other from Minnesota where we collect lymphoid tissues (LT) and peripheral blood monocytes (PBMCs) before and after vaccination using a new technique to catalog infectious burden of the individual, determine the relationship between IA, Infections, and immune response.
Objectives:	Primary Objective: To determine the relationship between endemic infections, immune activation (IA), T cell zone (TZ) fibrosis with loss of the fibroblastic reticular cell network (FRCn), CD4 and CD8 T cell subsets and the magnitude and durability of neutralizing antibody response to YFV in a cohort shown to have elevated IA, a damaged FRCn, and pan T cell depletion and a cohort that does not.
Endpoints:	Primary Endpoints: 1) Peak titer of neutralizing antibody to yellow fever vaccination

	Secondary Endpoints: 1. Measures of T cell zone fibrosis (collagen and desmin) in lymphatic tissue 2. Measures of past and present infection (pathseq, molecular analyses of helminth infection, serum antibody studies) in lymphatic tissue 3. Measures of immune activation (Ki67+ and CD69 + cells) in lymphatic tissue 4. Measures of systemic and lymphatic inflammation (plasma cytokines, frequency of TGFß+ and TNF+ cells in lymphoid tissues) in lymphatic tissue 5. Histologic analyses of lymphoid tissues for T follicular helper cells, changes in B cell populations, measures of BAFF+ cells, changes to B cell follicles. 6. Size and change to the frequency of yellow fever antigen specific CD4+ T cell population in lymphatic tissues. 7. Changes in CD8 T cell number and function in lymphatic tissues measured with transcriptomic analyses and standard functional assays (stimulation assays).
Study Population:	This study will include two cohorts of healthy adults in Kampala, Uganda and in Minnesota, USA. The cohort in Uganda will be 30 adults (15 men and 15 women) and the cohort in Minnesota will be 16 adults (8 men and 8 women).
Phase:	N/A
Description of Sites/Facilities Enrolling Participants:	Participants in Minnesota will be recruited and enrolled at the University of Minnesota, Minneapolis, MN. Study visits will occur in the University of Minnesota Health Clinical Research Unit. Participants in Kampala, Uganda will be recruited and enrolled at the Joint Clinical Research Center.
Description of Study Intervention:	Yellow fever vaccine (YFV) is a live-attenuated virus vaccine. It is administered in a single subcutaneous injection of 0.5mL of reconstituted vaccine.
Study Duration:	60 months
Participant Duration:	18 months

1.2 SCHEMA


Study N= 46
Day -42 to Day 1 (Screening)
Day -35 to Day 1 (Baseline)
Leukapheresis
LN Tissue bx
Luminex
Stool and urine collection
PBMC/plasma storage
T Cell Panel
Day 1
YFV Administration
Day 3 to Day 17 (Seen Every 2- 3 days)
YF Neutralizing Antibody
PBMC/Plasma storage
Blood draw (Day 17)
Stool collection (Day 10)
Week 3
LN Tissue bx
T Cell Panel
Luminex
PBMC/plasma storage
Weeks 4 – 10 (Seen Every 2 Weeks)
YF Neutralizing Antibody (week 8)
PBMC/Plasma storage
Stool collection (week 4)
Week 12
Stool and urine collection
HIV antibody
Luminex
PBMC/Plasma storage
Months 6 - 18
Seen Every 3 Months
stool and urine collection
Luminex (month 12)
PBMC/Plasma storage
HIV antibody
Month 6
Leukapheresis
Month 18- Final Visit
Stool and urine collection
HIV antibody
YF Neutralizing Antibody
Luminex
PBMC/Plasma storage

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Screening Day -42 to 1	Baseline Day -35 to 1	Day 1	Day 3 (+/- 1 day)	Day 5 (+/- 1 day)	Day 7 (+/-1 day)	Day 10 (+/-1 day)	Day 12 (+/-1 day)	Day 14 (+/-1 day)	Day 17 (+/-1 day)	Week 3 (+/-1 day)	Week 4 (+/- 3 days)	Week 6 (+/- 3 days)	Week 8 (+/- 3 days)	Week 10 (+/- 3 days)	Week 12 (+/- 3 days)	Month 6 (+/- 14 days)	Month 9 (+/- 7 days)	Month 12 (+/- 7 days)	Month 15 (+/- 7 days)	Final Study Visit Month 18 (+/- 7 days)
Informed consent	X																				
Physical exam (including height and weight)		X																			
Medical history	X	X																			
Urine Collection		X														X			X		X
Stool Collection		X [5,6]					X[5]					X[5]				X[6]	X[5]		X[5,6]		X[5,6]
CBCD/Plts	X																				
Chemistry Panel[3]	X																				
T Cell Panel	X	X									X										X
PT/PTT	X																				
HIV antibody	X															X			X		X
YF Neutralizing Ab	X			X		X		X		X				X							X
Luminex		X									X					X			X		X
LN Biopsy		X									X										
YF Vaccine			X																		
Leukapheresis[8]		X															X				
PBMC/Plasma Storage	X[1]	X		X	X	X	X	X	X	X[4]	X	X	X	X	X	X	X	X	X	X	X
Serum or urine pregnancy test[2]	X	X				X			X		X	X					X				
COVID-19 screening[7]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE Assessment		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

1. Plasma to screen for absence of yellow fever antibodies
2. For females of childbearing potential
3. Chemistry Panel to include Albumin, ALT, AST, Alk. Phosphate, Bilirubin (total), Calcium, Chloride, CO2, Creatinine, Glucose, Potassium, Protein (total), Sodium, Urea Nitrogen
4. This blood draw will be 60 cc for research
5. Samples will go to the Klatt lab
6. Samples will go to the Nutman lab
7. Verbal screening related to symptoms and recent exposures (see SOP for details)
8. Large volume blood draw (60 cc) will be done if a participant is not eligible for leukapheresis

2 INTRODUCTION

2.1 STUDY RATIONALE

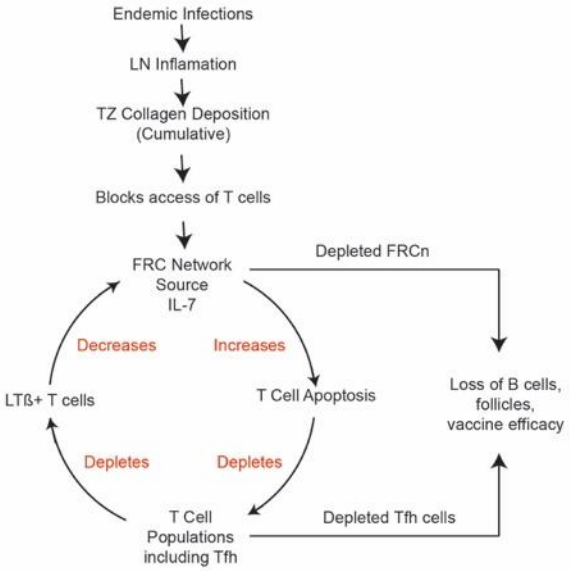
Vaccine responses are known to vary geographically. Bacillus Calmette-Guerin (BCG) for tuberculosis (TB) is perhaps the best known example of a vaccine where efficacy rates vary according to geography, with better responses occurring in northern latitudes (1). Rotavirus vaccine is another where sustained, neutralizing antibody titers are achieved after a single dose in the U.S. and Europe but in developing countries protection is absent or short-lived, even after multiple doses (2-4). Polio and cholera, are also associated with poor antibody responses in developing countries (5-11) and it was recently shown that yellow fever vaccine (YFV) was associated with low levels of neutralizing antibodies in Uganda compared to Switzerland with heightened immune activation (IA) in Ugandans found to be an important factor limiting vaccine responses (12).

Reasons for these geographic differences in vaccine response are unknown, but one possible explanation is that local environmental conditions or endemic infections affect immune function. HIV is an obvious example where infection decreases immune function because of loss of CD4 T cells. There are multiple mechanisms that contribute to CD4 T cell loss in HIV infection, however the major one is from virus replication in lymph nodes (LN) causing an inflammatory reaction, mediated by increased expression of TGFß from T-regulatory cells(13), that leads to collagen formation in the parafollicular T cell zone (TZ)(14). This fibrosis replaces the fibroblastic reticular cell network (FRCn), a structure that is vital to normal immune function. Loss of the FRCn leads to depletion of IL-7(15), a cytokine made by the FRCn, and important for T cell homeostasis. As collagen increases, IL-7 levels decrease and T cell apoptosis increases (15, 16). It is unknown if this process of LN fibrosis leading to lower CD4 counts occurs with other infections, however in large population-based studies of HIV negative people in East Africa, the average CD4 T cell count is significantly less than in Northern European populations. In Uganda, the mean CD4 T cell count is 754 cells/mm3 for men and 894 cells/mm3 for women (17) and in Ethiopia the average for the entire population is 667 cells/mm3 but in Denmark it is 1067 cells/mm3 (18). There are similar studies from other parts of the world that show the same trend of higher CD4 T counts the further north or more developed the country (19-21).

Our hypothesis is that infections other than HIV can cause LN inflammation and collagen damage to the FRCn which will lead to CD4 T cell depletion and impaired vaccine responses. This hypothesis is supported by our preliminary data documenting high levels of collagen in the LN TZ of HIV negative Ugandans (compared to the U.S.) with concomitant loss of the FRCn, the magnitude of which correlates to peak yellow fever neutralizing antibody titer after vaccination. We believe that infections common in the developing world (e.g., helminths, TB, Malaria, and Salmonella) can cause LN inflammation that leads to chronic immune activation (IA) and collagen deposition in the FRCn with subsequent loss of T cells, (including T follicular helper cells). The loss of the FRCn impairs humoral responses to vaccination. We propose a direct test of this hypothesis by studying yellow fever vaccine (YFV) in two cohorts of people, one from Uganda and the other from Minnesota where we collect lymphoid tissues (LT) (i.e., lymph node biopsies), and PBMCs before and after vaccination and using a new technique to catalog infectious burden of the individual, determine the relationship between IA, Infections, and immune response. We will build on our previous work and study larger cohorts of people with more frequent collection of samples.

A central question in vaccine research is why vaccine efficacy rates vary with good protection in some parts of the world and poor protection in others. For BCG, there is a clear geographic difference in efficacy with vaccination in northern latitudes achieving greater efficacy rates (1). Our preliminary data suggest one possible explanation is that inflammation from chronic endemic infections damages LN structures that are required for robust immune responses. We have previously demonstrated that HIV replication in LN causes inflammation that directly leads to CD4 T cell depletion by a process of fibrosis replacing the FRCn of the TZ. The FRCn produces IL-7, which is a primary homeostatic mechanism supporting LT CD4 and CD8 T cells but the FRCn is also important for B cell function and follicle formation. Loss of the FRCn directly leads to increased apoptosis of T cells, and all of their subsets (15, 16), decreased B Cell Activating Factor (BAFF), fewer follicles, and decreased responses to vaccination (22). As described below we now have evidence to suggest that other infections may cause LN fibrosis leading to decreased CD4 T cells which, in turn, may limit vaccine responses. This model is described in **Fig.1**. Demonstration that this process occurs with infections beyond HIV and that it limits vaccine efficacy is highly significant and would suggest that alternative vaccine constructs or strategies may be necessary to improve vaccine efficacy rates, especially for infections like HIV, Malaria, and TB where the greatest burden of infection is in the developing world.

Fig. 1: Proposed mechanism for LN inflammation and TZ fibrosis leading to impaired vaccine responses.



2.2 BACKGROUND

We have previously demonstrated an indirect and significant relationship between TZ fibrosis and CD4 T cell loss by a mechanism of FRCn damage (14-16, 23). This finding, along with the observation of lower T cell counts in developing countries (17-21) raised the possibility that infections other than HIV might also cause LN inflammation and fibrosis. To explore this possibility, we studied 3 groups of HIV negative people (**Table 1**). Group 1 included 30 people from Kampala, Uganda (16 women, median CD4 874 cells/µl) for assessment of baseline measures of inflammatory cytokines and LN fibrosis. A subset of 20 were then given YFV with follow-up LN biopsy 10-14 days after vaccination, a time-point when germinal center formation is at its peak (24, 25) and we then obtained blood samples over 14 months of follow-up to measure the titer and duration of YF neutralizing antibodies. Group 2 consisted of 10 HIV-negative

people from Minnesota, USA (3 women, median CD4 953 cells/µl) who did not receive YFV but where we did have LN samples to analyze TZ architecture concurrently with Group 1. Group 3 consisted of 10 HIV uninfected participants of a study (26) at Emory University in Atlanta, GA (4 women, CD4 unavailable) where they did receive YFV and where plasma samples from baseline before and again 2 weeks after vaccination were available, allowing direct comparison of antibody titers to Group 1. We also used previously published (23) LN collagen data from HIV+ people from the US as positive controls.

Group	Site	N	YFV	Samples	Duration FU
1	Uganda	30	Subset of 20	LN & blood before and after	14 months
2	Minnesota	10	No	LN	1 time-point
3	Emory University	10	Yes	Blood at baseline and 2 weeks after YFV	2 weeks

Table 1: Description of each group, which groups received YFV, and what samples were collected over FU

At baseline (prior to vaccination) HIV-negative Ugandans have elevated levels of immune activation (IA) and inflammation: We measured IL-1ß, IL-4, IL-6, IL-10, IL-17A, IL-21, IL-22, IL-23, IL-13, MIP-1β, TNF, TGFß, and IP10 in plasma samples from Group 1 (Uganda) and Group 3 (Emory) prior to YFV administration. We observed significantly higher levels of TGFß, IL-6, IL-4 and IL-21 and MIP-1ß in Ugandans ($p < 0.001$, $p = 0.03$, $p = 0.004$, $p = 0.001$, and $p = 0.01$ respectively**, Fig. 2A, B**). There were no other statistically significant differences detected. The increase in TGFß in Ugandans is interesting as it is the pro-inflammatory cytokine we have previously associated with TZ fibrosis (13) and may be an important mechanism for LN fibrosis in this population as well. We used immunohistochemistry (IHC) and quantitative image analysis (QIA) to stain for Ki67+ cells in LN as a measure of IA. Ugandans had significantly greater frequency of Ki67+ cells ($p = 0.0019$, Fig. 2C-E). Collectively these data demonstrate increased IA and inflammation in HIV negative Ugandans, especially in LN.

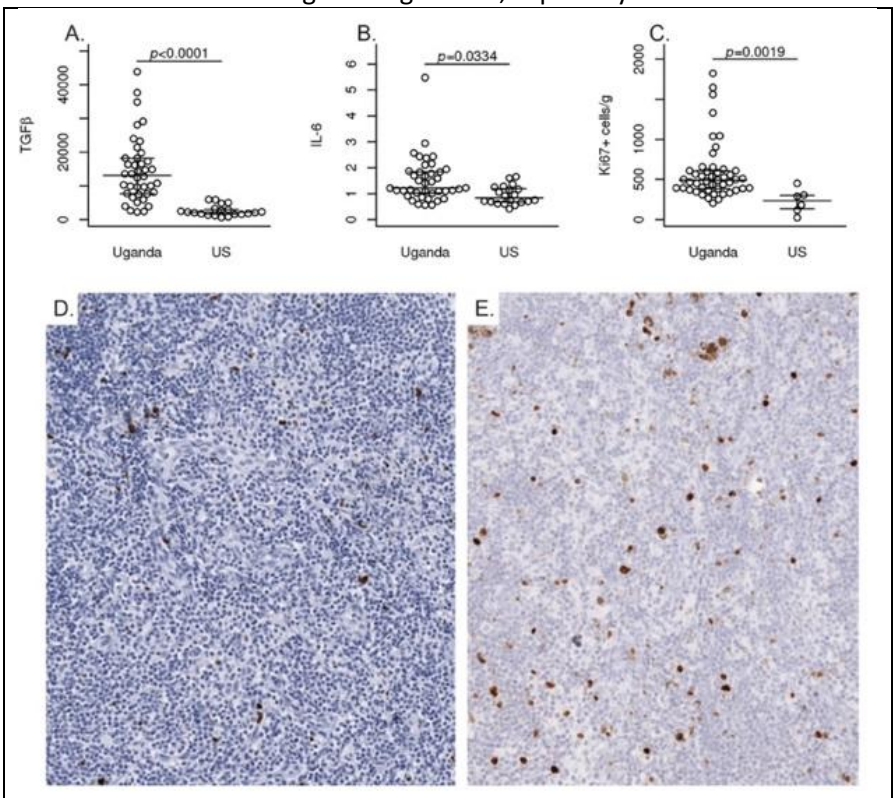


Fig. 2: Increased IA in HIV negative Ugandans. Increased levels of TGFß (A), IL-6 (B), and Ki67+ cells in LN (C) in Ugandans. LN sections stained with Ki67 from U.S. (D) and Ugandan I.

There is increased collagen and decreased CD4 T cells in HIV negative Ugandan LN. We used trichrome staining and QIA to determine the amount of collagen in the TZ in Groups 1 & 2 (**Fig. 3 A,B**). In Group 1 the mean area of the TZ with collagen was 12.4% (S.D. 3.4%) whereas in Group 2 it was 4.3% (s.d. 2.9%). The amount of TZ collagen in HIV negative Ugandans was similar to the HIV+ people from Minnesota where the mean amount of TZ fibrosis was 12.9% (s.d. 7.4%). The regression analysis, after log transformation of the percent TZ area, showed a significantly increased amount of TZ collagen in Group 1 compared to Group 2 ($p<0.0001$, **Fig. 3C**). We measured the size of the CD4 population in Group 1 and found the expected (14) significant inverse correlation between TZ collagen and TZ CD4 T cells ($p=0.0322$, **Fig. 3D**).

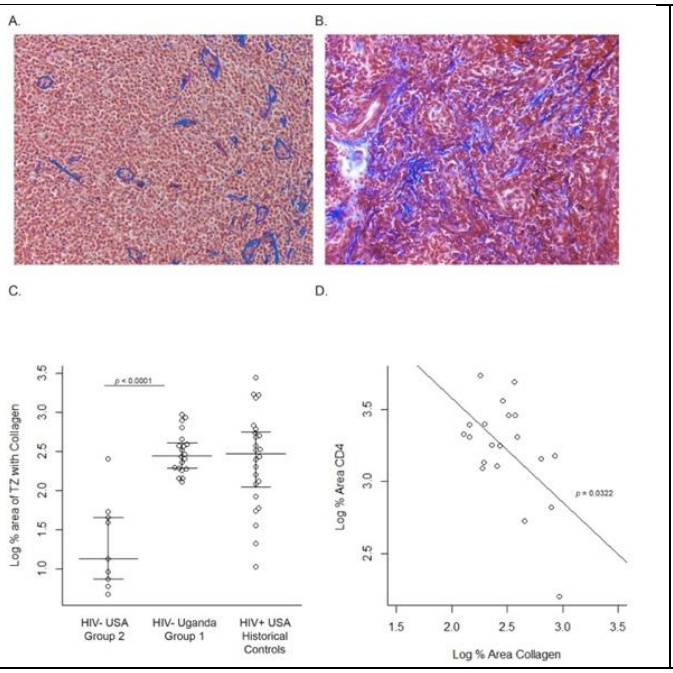


Fig. 3: Increased TZ fibrosis in Ugandans. Trichrome stain from a U.S. participant (A) and Ugandan (B)) shows increased collagen (blue fibers) in the Ugandan LN, similar to HIV+ people in the U.S. (C). There is the expected inverse relationship between TZ collagen and the size of the LN CD4 T cell population in Group 1 (D).

We next measured the FRCn by staining tissues with an antibody against Desmin. In **Fig. 4A** we show a representative section of the TZ of a LN from the Minnesota group where the Desmin positive TZ area was 22.6% whereas in the Ugandan Group it was 9.5% (range 5.1% - 19.4%, **Fig. 4B-C**). As expected, there was a direct and significant correlation between the log-transformed area of the TZ that was Desmin positive and the log-transformed size of the TZ CD4 T cell population ($p< 0.0001$, **Fig. 4D**).

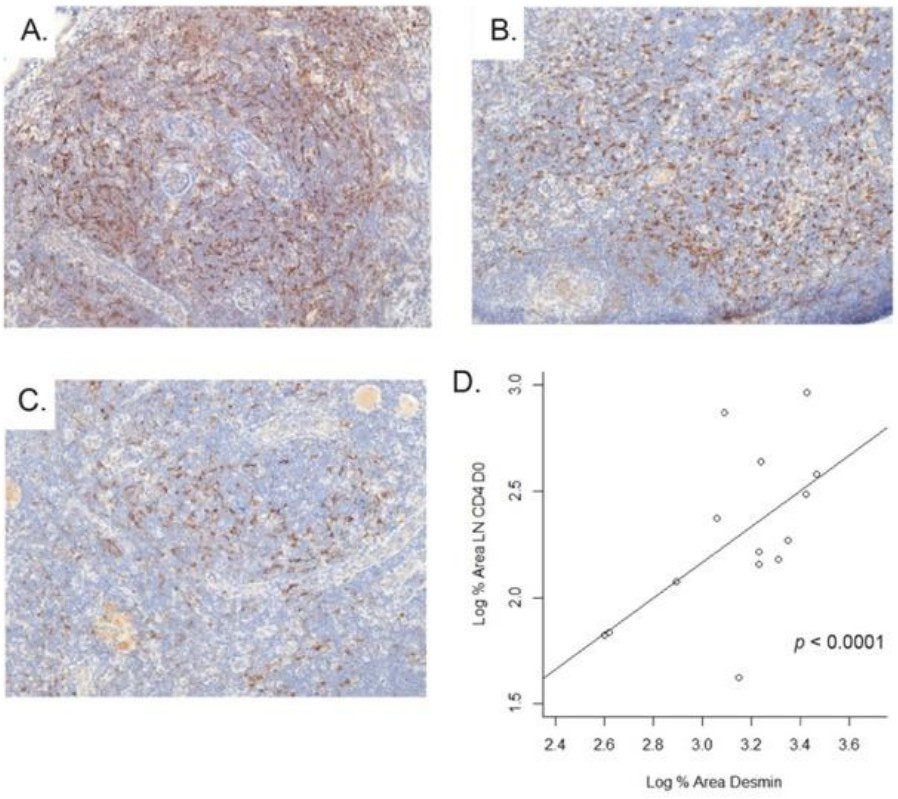


Fig. 4: The FRCn is depleted in Ugandans. We used QIA to identify TZ Desmin (brown) in HIV negative people in the U.S. (A) and Uganda (B and C) and then used quantitative image analysis to compare the amount of Desmin in the section to the size of the CD4 T cell population in the LN (D) showing the significant and direct relationship.

In summary, people in Uganda have increased IA, inflammation, TZ collagen, damage to the FRCn, and depletion of CD4 T cells. We next gave Group 1 YFV to determine if development of antibodies correlated to any measures of inflammatory damage to the LN.

There were few follicles prior to YFV and no changes in B cell follicles after YFV. We obtained an additional inguinal LN from 20 people in Group 1 and then administered YFV in the thigh opposite the one just biopsied. Ten to fourteen days later we obtained another LN from the same side the vaccine was given. We measured B cell populations, frequency and location of germinal centers, and frequency of Ki67+ cells before and after vaccination. We found few primary or secondary follicles (germinal centers) before vaccination with no significant increase after vaccination. In 63% of participants we saw no follicle formation and in only 3 of the participants did we see an increase in B cell follicles. The mean area of the follicles with B cells prior to vaccination was 25.5% (range 15.7-39.4%) whereas after vaccination it was 24.1% (range 14.0-34.1%, p=0.34). There was a significant increase in the frequency of Ki67+ cells (p=0.002).

YFV antibody responses were blunted and of short duration. Titers of neutralizing antibodies to YF were measured by plaque reduction assay (PRA). Initially we compared the week 2 samples between Group 1 and Group 3 using a PRA assay with a lower limit of detection of 10 plaque forming units (pfu) and found only a few of the Ugandans had detectable antibodies whereas most of Group 3 had detectable antibodies ($p < 0.0001$, **Fig. 5A**). We point out that we only had access to plasma samples from Group 3

at the 2 time-points (i.e., D0 and W2) and LN biopsies were not done in that study, so our comparative analysis of antibody formation and decay between these two groups is limited. However, as described below there are historical controls that can be used with Group 1 to make relevant comparisons about peak titer and decay rate(12). We next studied the longitudinally collected plasma samples from Group 1 using a PRA assay with a limit of detection of 100 pfu. We switched to a PRA with a higher threshold of detection titer because the lab performing the first analysis was no longer available to us. We did not duplicate the analysis between Group 1 and 3 as there was limited sample available. Peak titer was seen at the week 3 time-point with a 1.3 log range and a median decrease of 0.9 log by week 60 (**Fig. 5B**).

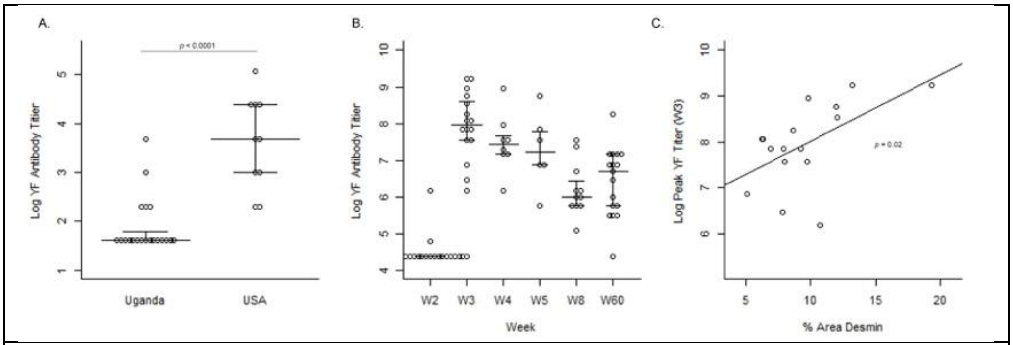


Fig. 5: Yellow fever antibody titers. Titers of neutralizing antibody from Groups 1 & 3 are compared using an assay with a lower limit of detection of 20 pfu (A). B. decay of antibodies through Month 14. C correlation of Desmin to peak antibody titer.

These response rates and decay characteristics are similar to those reported by Muyanja in a similar group of Ugandans (12) (and personal communication L. Trautmann, 2017). Given the variable response in antibody formation and the anatomic changes we found in Ugandan LN, we looked for an anatomic correlate of poor vaccine response and found that measures of the FRCn correlated to the peak antibody titer; the more FRCn, the higher the peak titer of antibodies (p = 0.02, **Fig. 5C**).

T Follicular Helper (Tfh) cells are depleted in HIV-negative Ugandans and do not increase with YFV. The striking fact that along with the diminished antibody response, we did not see an increase in follicles or B cell numbers we thought it possible that T Follicular helper cell (Tfh) populations might be diminished (as part of the overall CD4 T cell decrease) as they are required for normal B cell function and germinal center formation(27). We used a multiplexed confocal imaging assay allowing for the simultaneous detection of 6 markers in LN before and after vaccination with antibodies directed against CD20 for B cells, CD8, Ki67, and PD1 and CD57 for Tfh cells (28-30). We observed 3 distinct histologic patterns in these tissues. After vaccination only 3/20 had lack of Ki67 staining (Participant 1688, **Fig. 6K,L**). Ugandans exhibited a skewed cytokine response to YFV vaccination. We next measured IL-1ß, IL-4, IL-6, IL-10, IL-17A, IL-21, IL-22, IL-23, IL-13, MIP-1β, TNF, and TGFß in the plasma 2 weeks after YFV for comparison to the baseline measures discussed below (**Table 2**). In Group 3 (Emory) there was no change in any of the analytes between baseline and week 2 after vaccination other than IL-13, but we did measure significant changes in several cytokines in Group 1. There was a significant increase in IL-10, IL-17A, IL-21, IL-22, IL-23, IL-1ß, CXCL13, and IP10 in Ugandans after YFV (p > 0.01 for all). Levels of TGFß decreased significantly (p = 0.002) and there was a trend for decrease in TNF levels (p=0.065). The elevated levels of TGFß and IL-6 in the Ugandans prior to vaccination suggest prior exposures to extracellular pathogens or infections that promote IL-17 responses as evidenced by an increase in IL17A in the Ugandans after vaccination. The results of a lasso regression analysis show that IL6 and IL1b at Day 0 and IL22, TGFß and IL6 at W2 were predictive of yellow fever antibody titers at W3. Only IL1b was negatively correlated, the others were positively correlated.

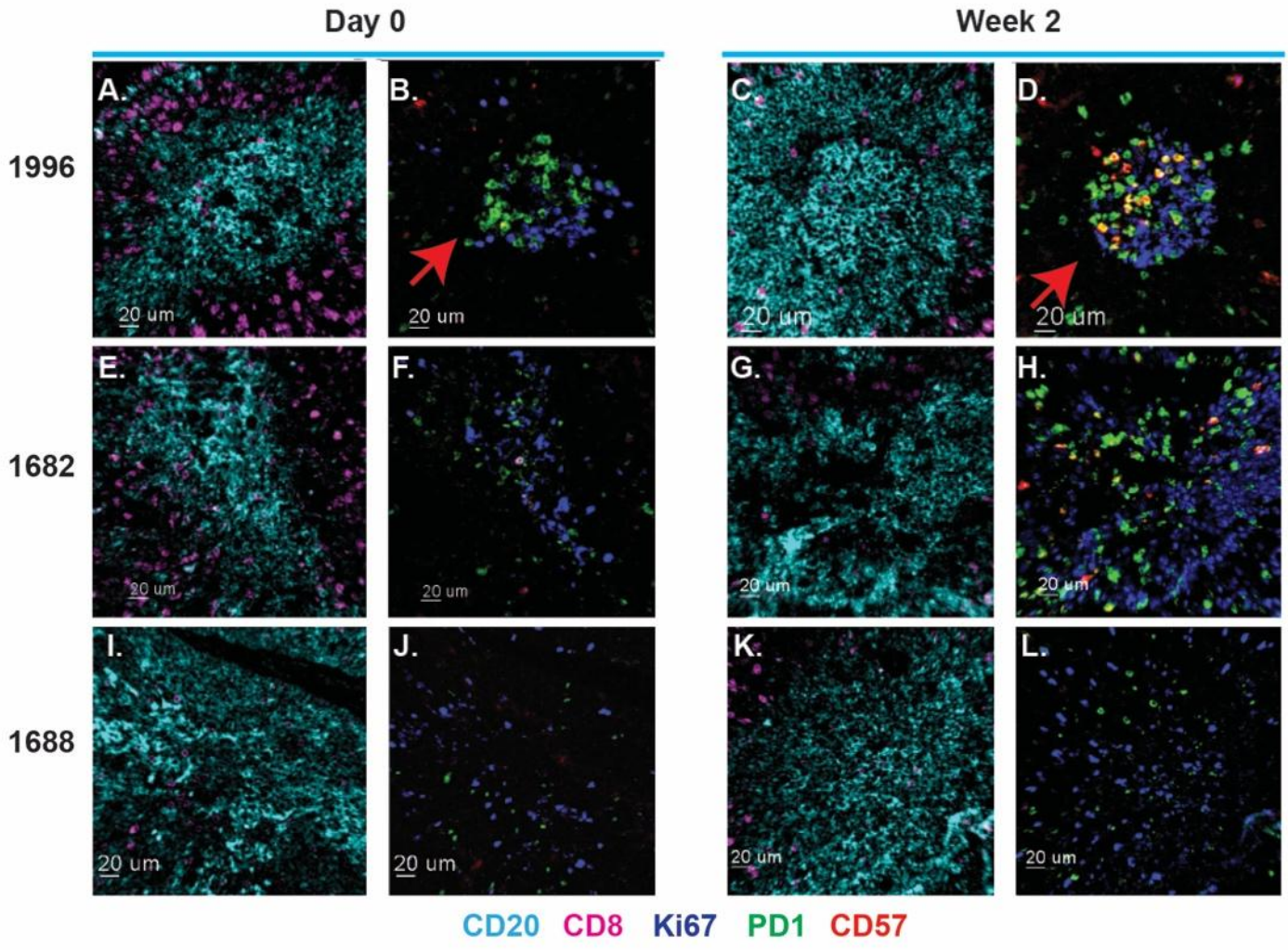


Fig. 6: B cell follicles are diminished with a paucity of Tfh cells in B cell follicles in Ugandans. This figure shows LN analyses from 3 different participants before vaccination and again at week 2 after vaccination. Participant 1996 has recognizable follicles pre-vaccination with maturing secondary follicles after vaccination (red arrows). The presence of PD-1 staining cells in the secondary follicle (green and blue staining cells in the secondary follicle appear yellow) is expected vaccination (D). Participant 1682 had fewer, more poorly formed follicles Day 0 and a lack of recognizable secondary follicles with vaccination. PD-1 staining cells are not inside of the follicle structure. Participant 1688 had no recognizable follicles prior to vaccination and no response to vaccination.

	–*p* - values							
	D 0 Uganda	W 2 Uganda	D 0 US	W 2 US	Uganda D0-W2	US D0-W2	Between Countries D0	Between Countries W2
TGFß	17768.7	11480.8	2464.6	2763	0.002	0.608	<0.001	<0.001
IL-13	17.4	22.7	10.2	8.5	0.107	0.013	0.083	0.002
IL-10	4.1	9	3.8	5.2	<0.001	0.063	0.646	0.179
IL-17A	33.6	51.1	35	37.3	0.004	0.31	0.889	0.053
IL-4	3.2	7	0.8	0.7	0.051	0.701	0.004	<0.001
IL-6	1.4	1.6	0.9	1	0.1	0.646	0.033	0.001
IL-21	1.5	3.3	<0.001	<0.001	0.006	1.0	0.001	<0.001
IL-22	0.5	3.2	<0.001	<0.001	0.022	1.0	0.362	0.008

IL-23	0.1	2.3	0.6	0.6	<0.001	0.781	0.588	0.012
IL-1ß	1.2	3.2	1.6	0.6	0.005	0.351	0.69	0.004
TNF	63.6	48.3	65.7	56.9	0.065	0.81	0.811	0.324
CXCL13	30.1	37.9	22.7	24.5	0.006	0.489	0.451	0.085
IP10	265	453.7	208.8	223.8	<0.001	0.107	0.062	<0.001
MIP1ß	57.6	49.3	36.8	35.4	0.065	0.137	0.01	0.077

Table 2: Summary of cytokine data in terms of models based estimates of mean levels. The models controlled for age, gender, and measurements over time from the same subject.

Our hypothesis is that infections cause inflammation and LN fibrosis. A particularly innovative feature of this protocol is the ability to characterize the infectious burden of an individual using the new "Pathseq" technique developed by Dr. Daniel Douek. This involves deep sequencing of RNA and DNA fragments (ranging in size from ~100-1000 bases) in plasma to obtain an overall quantitative and qualitative measure of the viral, bacterial, fungal, and parasite microbial burden as well as helminth burden in an individual. This approach differs from standard microbiome approaches such as bacterial 16s rDNA sequencing or virus purification and sequencing as it is sequence agnostic and takes advantage of the fact that circulating nucleic acids are typically highly fragmented to provide information about the "circulating microbiome". Nucleic acids from all microbes, worms and even food are readily detected using our PathSeq approach, and likely represent recent history of non-human genomic burden as circulating nucleic acids are typically very short-lived.

The methodology itself is a validated adaptation of our standard cellular RNAseq approach. Peripheral blood is collected in EDTA tubes, with one tube from the same batch remaining empty and processed identically as a control for contaminating nucleic acids. 1-10 ml plasma is separated and RNA and DNA are extracted. For the RNA, double-stranded cDNA is generated using random primers, and is then fragmented, end-repaired and dA-tailed. For the DNA, the protocol begins at the fragmentation step. Samples are cleaned with SPRI beads, and Illumina adaptors and barcodes ligated. A PCR enrichment is performed followed by low-cut purification to remove adaptor dimers. Libraries are now ready for QC, quantification and pooling. The control sample should show no detectable product at this stage and a positive signal would imply environmental contamination. Depending on the number of samples pooled, sequencing is then performed using Illumina MiSeq or HiSeq4000 instruments in the Douek lab. Following pre-processing of the raw sequences, host genomic and transcriptomic derived sequences are identified and removed from the dataset. De novo assembly is then performed on the remaining, non-host sequences. The assembled sequences are then aligned against the comprehensive "nt" database from NCBI to determine from which organism the assembled sequences were derived. The original, raw sequences are then mapped back to the assembled sequences to quantify the abundance of the assembled sequences and then, by proxy, the abundance of the organism from which the assembled sequences are derived. We assume the sensitivity of this approach is ~100% as our bio-informatic pipeline uses the most up-to-date microbial sequence databases but also corrects for inherent errors in these databases due to sequence contamination. In **Fig. 7** we show the PathSeq results on serial plasma samples collected from one Ugandan HIV+ individual over a 9-month period after starting antiretroviral therapy (ART). The y-axis shows fragments per kilobase of transcript per million reads (FPKM) normalized for total reads in each sample to allow for comparisons across time-points and subjects. The HIV reads become undetectable by 3 months of treatment. Bacteria account for the greatest number of reads with proteobacteria (predominantly gram-negative bacteria) being the largest component detected, presumably translocating from the gut. Helminths (both flat and round worms) also contributed substantially to the infectious burden. The Apicomplexan reads are predominantly derived from Plasmodium falciparum and the ssRNA virus reads are entirely derived from GBV-C virus. Importantly, the number of HIV reads and GBV-C reads were confirmed directly by specific qRT-PCR and

were completely concordant, providing further validation of this technique to assess the microbial burden. Also of importance, the pattern is remarkably stable over a 9-month period, suggesting patterns that emerge can be viewed as a chronic pattern of microbial burden.

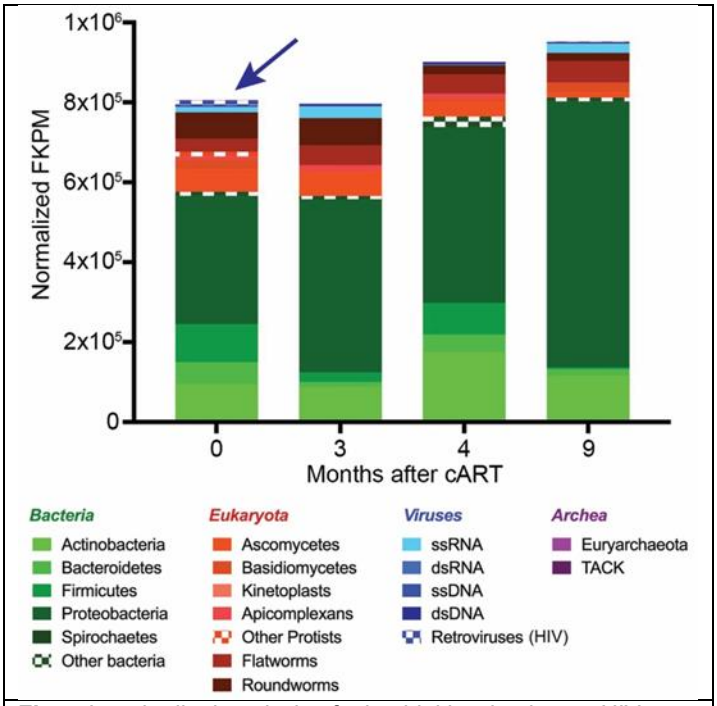


Fig 7: Longitudinal analysis of microbial burden in one HIV infected persons. Each bar represents the microbial burden at a different time-point over 9 months of follow-up. Bacteria make up the majority of reads detected and the pattern is remarkably stable over time. The blue arrow points to the blue checkered band that represents HIV. This individual started ART at that time and this band diminishes over time (as would be expected).

In **Fig. 8** we show the PathSeq result from 10 Ugandan HIV+ individuals collected over 9 months after initiating ART. The y-axis shows relative read frequency. We noted two distinct patterns of infectious burden. The plasma of the 6 participants in the upper row of **Fig. 8** predominantly contained bacterial nucleic acids from the Proteobacteria family whereas in the lower row of 5 participants GBV-C virus and helminthic nucleic acids predominated. Each group exhibited a markedly different cytokine profile after 9 months of ART. Infection with primarily proteobacteria was associated with higher levels of the pro-inflammatory cytokines IL-6 and TNF, and sCD14 (a marker of bacterial translocation) and inversely correlated to IL-17 and MIP1b. In contrast, participants with predominantly helminthic and GBV-C nucleic acids had exactly the opposite cytokine profile after 12 months of ART; high levels of IL-17 and MIP1b and low levels of IL-6 and sCD14.

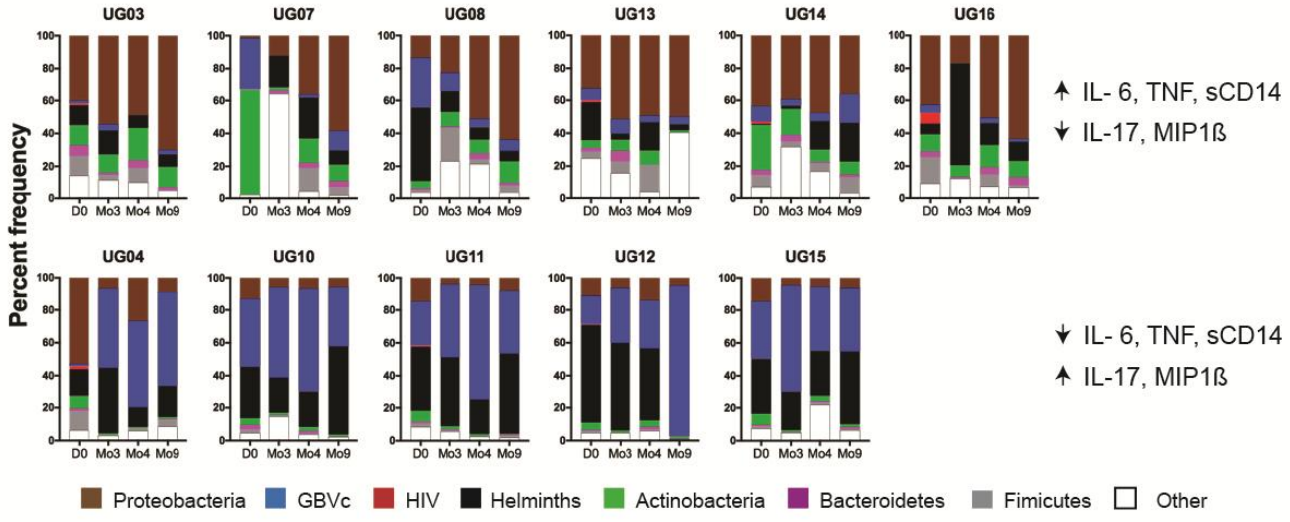


Fig 8: Two patterns of microbial burden identified over 9 months of FU.

Pathseq is a tool to provide quantitative information about the chronic infectious burden of an individual that we can use to investigate relationships between infections, LN inflammation and fibrosis, and vaccine responses. There is evidence that such relationships likely exist. For example, in a recent HIV vaccine study in Africa, participants infected with S. mansoni had significantly lower titers of antibodies than those who were not (31). Combining Pathseq with our analyses of antibody titers, cytokine profiles before and after vaccination, and our analysis of LN architecture will provide important information about the role of the local infectious burden as a determinant of immune response to vaccination.

We show in our preliminary data that chronic IA, inflammation and damage to LN architecture in HIV negative Ugandans correlates with neutralizing antibody titers after YFV. The demonstration of increased levels of TGFß at baseline along with an IL-10 and IL-17 response after YFV suggests helminthic infections may be contributing to abnormal vaccine responses and supports our hypothesis that endemic infections may affect immune responses to vaccination. We demonstrate the ability to characterize infectious burden, and while this does not define the entire life history of pathogen exposure, it is surprisingly stable over time allowing us to use this as a record of infectious exposures. We now propose to build on these initial observations by conducting a controlled study with YFV in both Uganda and the USA to better understand how endemic infections and environmental factors may affect innate and adaptive immune responses to vaccination and better define how these factors might inhibit immune mechanisms and components that lead to sustained immunity. Our data raise several important questions, including 1) what is causing the generalized IA, 2) how does systemic IA lead to a local process of collagen formation in the FRCn, 3) how are antigen specific CD4 and CD8 T cells affected by loss of the FRCn, 4) Why are their fewer TFh in Ugandans, 5) does the frequency and function of TFh cells in Ugandans affect the magnitude and durability of the vaccine response, 6) does relative T cell depletion in this population limit recall responses, and 7) does the mucosal environment, in particular in GALT infections from helminths or enteric pathogenic bacteria, affect the magnitude or durability of immune responses? Our hypothesis is that infections other than HIV can cause LN inflammation and collagen damage to the FRCn which will lead to CD4 T cell depletion and impaired vaccine responses.

We will address this hypothesis by giving YFV to a group of otherwise healthy people in Uganda and Minnesota and we will systematically collect blood, and LN specimens before and after YFV. Our approach is feasible as we have already demonstrated that we can conduct a trial of this nature at the

Joint Clinical Research Center in Kampala, Uganda where we have collected LN and blood samples at multiple time-points throughout several protocols, including the YFV study described above. In the past 8 years, we have enrolled over 105 participants in HIV pathogenesis and vaccine studies at the JCRC who have contributed 228 LN biopsies. Importantly, we now have capacity to perform leukapheresis at the JCRC in Uganda as they recently acquired the equipment and expertise to perform this procedure. We have demonstrated the ability to process, maintain, and ship specimens from the Uganda site to Minnesota. In Minnesota, we have enrolled 170 HIV+ and 100 HIV- people in multiple pathogenesis and interventional trials where we have collected 413 LNs, 1657 PBMC and plasma samples from these individuals. We now propose to collect the relevant specimens before and after YFV to apply state-of-the-art technologies to address fundamental question about how and why vaccine responses are limited in some populations.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Risks Associated with Yellow Fever Vaccine

Common side effects:

- Injection site reaction, including erythema and pain at injection site
- Headache
- Fever
- Myalgia
- Malaise

Rare side effects:

Severe Allergic Reactions

Severe allergic reactions (e.g., anaphylaxis) may occur following the use of Stamaril or YF-VAX, even in individuals with no prior history of hypersensitivity to the vaccine components. Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of the vaccine.

Yellow fever vaccine-associated viscerotropic disease

Age greater than 60 years is a risk factor for yellow fever vaccine-associated viscerotropic disease (YEL-AVD) which may present as non-specific multi-organ system failure or can be similar to fulminant yellow fever caused by wild-type yellow fever virus, with liver failure and internal bleeding, leading to death. Available evidence suggests that the occurrence of this syndrome may depend upon undefined host factors, rather than intrinsic virulence of the yellow fever strain 17D vaccine, based on characterization of vaccine viruses isolated from individuals with YEL-AVD. YEL-AVD has been reported to occur only after the first dose of yellow fever vaccine; there have been no reports of YEL-AVD following booster dose. The decision to vaccinate individuals 60 years of age and older needs to weigh the risks and benefits of vaccination and the risk for exposure to yellow fever virus.

Yellow fever vaccine-associated neurotropic disease

Age greater than 60 years and immunosuppression are risk factors for post-vaccinal encephalitis, also known as yellow fever vaccine-associated neurotropic disease (YEL-AND). Almost all cases of YEL-AND have been in first-time vaccine recipients. The decision to vaccinate individuals 60 years of age and older

and immunosuppressed individuals needs to weigh the risks and benefits of vaccination and the risk for exposure to yellow fever virus.

Risks Associated with Protocol Directed Procedures:

Lymph Node Biopsy

The risks of the lymph node biopsy are bleeding, infection, seroma, and scarring. Any participant who develops a seroma will have it drained with a syringe in the clinic. These complications are rare. All post-operative and post-biopsy care will be provided by study nurses. Participants will be monitored for a minimum of 4 hours post-surgery prior to being allowed to return home. If complications occur, the participant will be seen immediately by a qualified surgeon.

Leukapheresis

The risks of the apheresis process include bruising and/or bleeding in the arms when needles are placed for the apheresis; a reaction to the drug used as an anticoagulant during the procedure, citrate; and loss of blood, especially platelets. Rarely, veins cannot be found to insert the needles a catheter must be placed in a large neck vein to collect the cells.

Blood Draw

Events associated with venipunctures include discomfort, slight bruising, bruising, bleeding, lightheadedness, fainting, infection at the venipuncture site, nausea, anxiety and swelling at the venipuncture site.

2.3.2 KNOWN POTENTIAL BENEFITS

There are no anticipated benefits to participants on this study.

There is a potential for societal benefit, as this study could demonstrate the need for alternative vaccine constructs and other strategies to improve vaccine efficacy rates.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

There is a low possibility of side effects. There are no potential benefits besides findings from the proposed research could lead to a clearer understanding of the requirements for inducing durable protective immunity.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine the relationship between endemic infections, immune activation (IA), T cell zone (TZ) fibrosis with loss of the fibroblastic reticular cell network (FRCn), CD4 and CD8 T cell subsets and the magnitude and durability of neutralizing antibody response to YFV in a cohort shown to have elevated IA,	Peak titer of neutralizing antibody to yellow fever vaccination	The primary analysis endpoint is the peak titer of neutralizing antibody. We believe that there will be significant differences in the peak titer and durability of titers between the two groups.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
a damaged FRCn, and pan T cell depletion and a cohort that does not.		
Secondary		
To determine predictors of levels of neutralizing antibodies	1. Measures of T cell zone fibrosis (collagen and desmin) 2. Measures of past and present infection (pathseq, molecular analyses of helminth infection and serum antibody studies) 3. Measures of immune activation (Ki67+ and CD69 + cells) 4. Measures of systemic and lymphatic inflammation (plasma cytokines, frequency of TGFß+ and TNF+ cells in lymphoid tissues). 5. Changes in T cell number and function	We believe the reason that titers will be different is that inflammation from endemic infections alters the architecture and therefore the function of the lymphatic tissues which results in less antibody being made. With these secondary endpoints we will measure these potential predictors.
Tertiary/Exploratory		
To determine the impact of LN fibrosis on specific T cell populations including T follicular helper cells and antigen specific T cells.	1. Histologic analyses of lymphoid tissues for T follicular helper cells and B cells. 2. Size and change to the frequency of yellow fever antigen specific CD4+ T cell population.	We have shown that lymphatic tissue fibrosis causes depletion of CD4 and CD8 + T cells however we have never examined the impact on highly specific subsets.

4 STUDY DESIGN

4.1 OVERALL DESIGN

The primary aim of this study is to determine the difference between antibody titers in the two study groups and study the relationship between endemic infections, IA, the FRCn, and CD4 and CD8 T cell subsets and the magnitude and durability of neutralizing antibody response to YFV in a cohort shown to have elevated IA, a damaged FRCn, and pan T cell depletion and a cohort that does not. This is a single arm, open-label, two cohort study of healthy adults in Kampala, Uganda and in Minnesota, USA. The cohort in Uganda will be 30 adults (15 men and 15women) and the cohort in Minnesota will be 16 adults (8 men and 8 women). Everyone will be screened to ensure there are no contraindications to receiving YFV (e.g., immunosuppression) or the planned procedures. The inclusion and exclusion criteria are discussed in detail in the protocol that is included in the appendix. Participants will have an inguinal LN biopsy and leukapheresis prior to YFV and another LN biopsy 3 weeks after the vaccine administration. The vaccine will be given in the contralateral thigh from the first LN biopsy so that the second biopsy will be from a draining LN. PBMC and plasma will be collected at regular intervals over the 18-month follow-up period. Leukapheresis will be done again at the month 6 visit.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Our preliminary data shows that lymph node inflammation and damage to the FRCn correlates to peak titer. We also show data to suggest that there is significantly less LN damage from inflammation in native Minnesotans (not immigrants—we have not studied that). We think the differences are because of exposure to endemic infections. We will therefore use an open-label, non-randomized design to test our hypothesis.

4.3 JUSTIFICATION FOR DOSE

This is the standard of care dose of yellow fever vaccine.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

Study entry is open to adults regardless of race or ethnic background. While there will be every effort to seek out and include minority patients from both genders, the patient population is expected to be no different from that of the populations in Minnesota and Kampala, Uganda.

In order to be eligible to participate in this study, an individual must meet all of the following criteria

1. Age $\geq$18 years but < 60 years
2. No contraindication to Yellow Fever vaccine (immunosuppressed for any reason or on an immunosuppressive drug where a live virus vaccine is contraindicated).
3. If female of childbearing age must agree to contraception for one month following administration of the vaccination.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study

1. History of yellow fever or previous vaccination for yellow fever
2. Known bleeding disorder
3. Prior surgery complicated by clotting abnormality
4. Psychiatric or behavioral disorder that, in the opinion of the investigator, will make it difficult for the participant to complete the study
5. History of acute hypersensitivity reaction to any component of the vaccine (including gelatin, eggs, egg products, or chicken protein).
6. Thymus disorder associated with abnormal immune function
7. Immunosuppression from any of the following: HIV infection or AIDS, malignant neoplasms, primary immunodeficiencies, transplantation, transplantation, immunosuppressive or

immunomodulatory therapy (corticosteroids, alkylating agents, antimetabolites, TNF inhibitors, IL-1 blocking agents, monoclonal antibodies targeting immune cells), previous radiation therapy.
8. Pregnant or breastfeeding at the time of vaccination.
9. Planning to conceive within 28 days of enrollment and vaccination with the yellow fever vaccine.

5.3 LIFESTYLE CONSIDERATIONS

N/A

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently eligible to be entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

In Minneapolis, Minnesota, participants will be recruited through the use of flyers via email, mail, and fax, study website, advertisements and primary care providers making information available to potential participants. In addition, the research team will work with the Fairview Uptown travel clinic to identify potential participants based on yellow fever vaccination appointments. In Kampala, Uganda recruiting will occur by referral from primary doctors and advertisements.

6 STUDY INTERVENTION

There are no study interventions and the following information is provided as they are central to the study. Stamaril and YF-VAX are the manufacturers and both can be used at either study site.

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

YF-VAX® and Stamaril®, Yellow Fever Vaccine, for subcutaneous use, is prepared by culturing the 17D-204 strain of yellow fever virus in living avian leukosis virus-free (ALV-free) chicken embryos. The vaccine contains sorbitol and gelatin as a stabilizer, is lyophilized, and is hermetically sealed under nitrogen. No preservative is added. Each vial of vaccine is supplied with a separate vial of sterile diluent, which contains Sodium Chloride Injection USP – without a preservative. Stamaril is formulated to contain not less than 4.74 log10 plaque forming units (PFU) per 0.5 mL dose throughout the life of the product. Before reconstitution, Stamaril is a pinkish color. After reconstitution, Stamaril is a slight pink-brown suspension. The vial stoppers for Stamaril and diluent are not made with natural rubber latex.

YF-VAX and Stamaril are approved for the prevention of yellow fever in persons 9 months of age and older.

Stamaril is an already licensed product in Uganda. It was approved by the National Drug Authority (NDA) under registration / reference number NDA/MAL/HDP/1482. The vaccine in manufactured by SANOFI PASTUER in France and is distributed by Laborex (U) Limited in Uganda.

6.1.2 DOSING AND ADMINISTRATION

YF-VAX and Stamaril are administered in a single subcutaneous injection of 0.5 mL of reconstituted vaccine.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

In Minnesota, the vaccine will be administered per the study's vaccine administration SOP at Park Nicollet Travel Clinic in St. Louis Park, MN, Fairview Uptown Travel Clinic in Minneapolis, MN, or any travel clinic that is authorized to administer the yellow fever vaccine. The travel clinician will indicate the vaccine administration site for study documentation.

In Uganda, the vaccine will be supplied from the commercial pharmacy and administered in the research clinic in a manner identical to what is described above in our background discussion.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

YF-VAX® and Stamaril®, Yellow Fever Vaccine, for subcutaneous use, is prepared by culturing the 17D-204 strain of yellow fever virus in living avian leukosis virus-free (ALV-free) chicken embryos. The vaccine contains sorbitol and gelatin as a stabilizer, is lyophilized, and is hermetically sealed under nitrogen. No preservative is added. Each vial of vaccine is supplied with a separate vial of sterile diluent, which contains Sodium Chloride Injection USP – without a preservative. Stamaril is formulated to contain not less than 4.74 log10 plaque forming units (PFU) per 0.5 mL dose throughout the life of the product. Before reconstitution, Stamaril is a pinkish color. After reconstitution, Stamaril is a slight pink-brown suspension. The vial stoppers for Stamaril and diluent are not made with natural rubber latex.

6.2.3 PRODUCT STORAGE AND STABILITY

Store at 2° to 8°C (35° to 46°F). DO NOT FREEZE. Do not use vaccine after expiration date. Stamaril does not contain a preservative. The following stability information for Stamaril is provided for those countries or areas of the world where an adequate cold chain is a problem and inadvertent exposure to abnormal temperatures has occurred. Half-life is reduced from approximately 14 days at 35° to 37°C to 3-4.5 days at 45° to 47°C.

6.2.4 PREPARATION

Reconstitute the vaccine using only the diluent supplied (0.6 mL vial of Sodium Chloride Injection USP for single dose vial of vaccine and 3 mL vial of Sodium Chloride Injection USP for 5 dose vial of vaccine). After removing the "flip-off" caps, cleanse the vaccine and diluent vial stoppers with a suitable germicide. Do not remove the vial stoppers or metal seals holding them in place. Using aseptic technique, use a suitable sterile needle and syringe to withdraw the volume of supplied diluent shown on the diluent label and slowly inject the diluent into the vial containing the vaccine. Allow the reconstituted vaccine to sit for one to two minutes and then carefully swirl mixture until a uniform suspension is achieved. Avoid vigorous shaking as this tends to cause foaming of the suspension. Do not

dilute reconstituted vaccine. Use aseptic technique and a separate sterile needle and syringe to withdraw each 0.5mL dose from the single dose or multidose vial of reconstituted vaccine.

Before reconstitution, the vaccine is a pinkish color. After reconstitution, the vaccine is a slight pink brown suspension. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, do not administer the vaccine.

Administer the single dose of 0.5 mL subcutaneously using a suitable sterile needle.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Investigators conducting image analysis will be blinded to which group the tissue they are analyzing is from (Uganda vs. Minnesota).

A special numbering system will be created by Drs. Helgeson and Reilly and used for all samples so that people conducting other analyses of samples will be blinded to which group the tissue they are analyzing is from (Uganda vs. Minnesota).

Only Drs. Reilly and Helgeson will have access to the identification information and will be able to match samples to group.

6.4 STUDY INTERVENTION COMPLIANCE

This is a single dose of vaccine. Compliance should not be an issue.

6.5 CONCOMITANT THERAPY

There are no medication restrictions on this protocol.

6.5.1 RESCUE MEDICINE

Not applicable.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

N/A

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Pregnancy within 28 days of vaccination
- Significant study non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant

- Acquisition of HIV

The reason for participant discontinuation or withdrawal from the study will be recorded on a Case Report Form (CRF) designed for this purpose. Subjects who sign the informed consent form but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

This study does not contain a therapeutic intervention and therefore there are no efficacy assessments.

8.2 SAFETY AND OTHER ASSESSMENTS

Screening Procedures (Day -42 to 1)
Participants will be consented by the investigator or qualified study staff before any procedures take place.

The following screening blood tests will be obtained:

- CBCD
- Chemistry Panel (including Albumin, ALT, AST, Alk. Phosphate, Bilirubin (total), Calcium, Chloride, CO2, Creatinine, Glucose, Potassium, Protein (total), Sodium, Urea Nitrogen)
- T Cell Panel
- PT/PTT
- HIV antibody
- Yellow Fever Neutralizing Antibody
- PBMC/Plasma storage (to screen for yellow fever)

We will obtain a detailed medical history to ascertain that potential subjects have not had yellow fever, been vaccinated for yellow fever, and have no history of thymus disease. Females of childbearing

potential will have a serum or urine pregnancy test. During the COVID-19 pandemic, participants will be screened for COVID-19 symptoms before coming in for their visit.

Baseline Procedures (Day -35 to 1)
Medical history and concurrent medications ascertained. Physical examination and vital signs, including blood pressure, pulse and weight will be conducted. There will also be an AE assessment. Leukapheresis will be performed. Before Leukapheresis, females of childbearing potential will have a serum or urine pregnancy test. If a participant is not eligible for leukapheresis based on the apheresis consultation appointment or is unable to tolerate leukapheresis, a large volume blood draw (60 cc) will be done instead. Participants will undergo a lymph node biopsy tissue will be stored. Participants will have stool and urine collected for a helminth check. Stool will be collected for microbiome study.
Blood will be collected for:

- T cell panel
- Luminex
- PBMC/plasma storage

During the COVID-19 pandemic, participants will be screened for COVID-19 symptoms before coming in for their visits.

Day 1
Yellow fever vaccine will be administered. During the COVID-19 pandemic, participants will follow the travel clinics' procedures for screening of COVID-19 symptoms before coming in for their visit.

Days 3 through 14
Participants will return every 2 to 3 days (see SOEs, Sections 1.2 and 1.3) for blood collection for:

- PBMC/plasma storage
- Yellow fever neutralizing antibody

Stool will be collected on Day 10 for microbiome study.

On Days 7 and 14, females of childbearing potential will have a serum or urine pregnancy test.

During the COVID-19 pandemic, participants will be screened for COVID-19 symptoms before coming in for their visits. There will also be AE assessments.

Day 17
At Day 17, participants will have a research blood draw of 60 cc. Blood will be collected for yellow fever neutralizing antibody. There will also be an AE assessment. During the COVID-19 pandemic, participants will be screened for COVID-19 symptoms before coming in for their visit.

Week 3
At Week 3, participants will undergo a lymph node biopsy will be stored. Blood will be collected for:

- T cell panel
- Luminex
- PBMC/plasma storage

Females of childbearing potential will have a serum or urine pregnancy test. There will also be an AE assessment. During the COVID-19 pandemic, participants will be screened for COVID-19 symptoms before coming in for their visit.

Weeks 4 through 10
Participants will return every 2 weeks (see SOEs, Sections 1.2 and 1.3) for blood draws for:

- PBMC/plasma storage
- Yellow fever neutralizing antibody (week 8 only)

At Week 4, females of childbearing potential will have a serum or urine pregnancy test. Stool will be collected for microbiome study.

There will also be an AE assessment. During the COVID-19 pandemic, participants will be screened for COVID-19 symptoms before coming in for their visit.

Week 12
Blood will be collected for:

- HIV antibody
- PBMC/plasma storage
- Luminex

Stool and urine will be collected to check for helminths. There will also be an AE assessment. During the COVID-19 pandemic, participants will be screened for COVID-19 symptoms before coming in for their visit.

Months 6 through 15
Participants will return quarterly starting Month 6 through Month 18.

Months 6, 9, 12 and 15 participants will have blood collected for PBMC/plasma storage.

Month 6 will have stool will be collected for microbiome study. During the month 6 timepoint, leukapheresis will be performed. Before leukapheresis, females of childbearing potential will have a serum or urine pregnancy test. If a participant is not eligible or unable to tolerate leukapheresis, a large volume blood draw (60 cc) will be done.

At the month 12 visit, participants will have stool and urine collected for helminth check. Stool will be collected for microbiome study, and blood will be collected for:

- HIV antibody
- Luminex
- PBMC/plasma storage

There will also be an AE assessment. During the COVID-19 pandemic, participants will be screened for COVID-19 symptoms before coming in for their visit.

Month 18
The month 18 visit will be the final study visit. Stool and urine will be collected for helminth check. Stool will be collected for microbiome study. Blood will be collected for:

- T cell panel
- HIV antibody
- Yellow fever neutralizing antibody
- Luminex
- PBMC/plasma storage

There will also be an AE assessment. During the COVID-19 pandemic, participants will be screened for COVID-19 symptoms before coming in for their visit.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

Toxicity and adverse events will be classified according to Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. A copy of this can be downloaded from the DAIDS home page at *https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf*

AE/SAE Grading Assignment

All adverse events will be graded in the following manner:

- Grade 1 (Mild): Events require minimal or no treatment and do not interfere with the participant's daily activities.
- Grade 2 (Moderate): Events result in a low level of inconvenience or concern. Moderate events may cause some interference with functioning.
- Grade 3 (Severe): Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

- Grade 4 (Life-threatening): Any adverse drug experience that places the participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred (i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death).
- Grade 5 (Death)

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

8.3.3.3 EXPECTEDNESS

The principal investigator will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The principal investigator or study staff will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of

AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

In Uganda, the sponsor has taken out clinical trials insurance policy to cover compensation for any personal injury resulting from participants taking the study medication and/or study procedures, provided such personal injury is not due to fault or negligence of the study doctor or his team.

8.3.5 ADVERSE EVENT REPORTING

Adverse events will be reported to the University of Minnesota Institutional Review Board annually, at the time of continuing review, in accordance with their reporting policy.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

All serious adverse events that meet the University of Minnesota Institutional Review Board's prompt reporting requirements will be reported to the IRB no later than 5 business days. All SEAs will also be reported to the JCRC IRB within 5 business days of awareness.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

Not applicable.

8.3.8 EVENTS OF SPECIAL INTEREST

Not applicable.

8.3.9 REPORTING OF PREGNANCY

Not applicable.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the University of Minnesota Institutional Review Board (IRB) and JCRC IRB per the UMN IRB's and JCRC IRB's prompt reporting requirements.

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Not applicable.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

- Primary Efficacy Endpoint(s):

Null hypothesis: There is no difference in peak yellow fever antibody titer between individuals from the US and Uganda.
Alternative hypothesis: There is a difference in peak yellow antibody titer.

- Secondary Efficacy Endpoint(s):

Null hypothesis: There is no difference in baseline LN architecture measures (TZ collagen, FRCn, B cell follicles and populations, Tfh cells, and B Cell activating factor) between individuals from the US and Uganda. Alternative hypothesis: There are differences in these measures between the two countries.

Null hypothesis: There is no association between baseline measures of LN architecture and rate of YFV antibody decay. Alternative hypothesis: There are associations between these measures and rate of antibody decay.

Null hypothesis: Principal components of pathseq data are not associated with country of origin. Alternative hypothesis: Some principal components of pathseq data are be associated with country.

Null hypothesis: There are no difference in helminth infection patterns between the US and Uganda. Alternative hypothesis: Differences in helminth infections exist between the two countries.

Null hypothesis: Pathseq principal components with strong loadings for infection organisms are not associated with baseline measures of LN architecture. Alternative hypothesis: PC components with strong loadings for infectious organisms are associated with baseline LN architecture measures.

Null hypothesis: Presence of helminth infection is not associated with baseline LN architecture measures. Alternative hypothesis: Presence of helminth infection is associated with baseline LN architecture measures.

Null hypothesis: There is no association between changes in immune function after vaccination and measures of LN architecture. Alternative hypothesis: There is an association between change in immune function and measures of LN architecture.

Null hypothesis: There is no association between CD4, CD8 functional variables and CD4 subsets and peak YFV antibody titers or rate of antibody decay. Alternative hypothesis: There is an

association between CD4, CD8 functional variables and CD4 subsets and peak YFV antibody titers and rate of antibody decay.

Null hypothesis: There is no association between CD4, CD8 functional variables and CD4 subsets and peak specific IgM, IgG1, IgG2, IgG3 and IgG4 responses or rate of change. Alternative hypothesis: There is an association between CD4, CD8 functional variables and CD4 subsets and peak specific IgM, IgG1, IgG2, IgG3 and IgG4 responses or rate of change.

Null hypothesis: There is no change in immune activation or inflammation measures or CD4 and CD8 cells after immunization. Alterative hypothesis: There is a change in immune activation or inflammation measures or CD4 and CD8 cells after immunization.

9.2 SAMPLE SIZE DETERMINATION

We will enroll 30 individuals from Uganda and 16 individuals from the US. With this sample size our study will have more than adequate power to test our primary aim, differences in peak antibody titers between countries. Assuming yellow fever antibody titers show a similar range as in our previous studies (a standard deviation of 1 log) for both populations we have over 85% power to detect a difference in peak antibody titers of 1 log, with a two-sided test with alpha level of 0.05. This calculation is based on comparison to a T distribution with 43 degrees of freedom.

Since, in fact, we will be using a Mann-Whitney U test for this comparison we also illustrate that this sample size is sufficient using power simulations. For all simulations we assume that the Ugandan population will have a mean of 7.89 log titers and approximate standard deviation of 1 and the Minnesota population has a mean of 8.89 and an approximate standard deviation of 1. One thousand iterations were conducted for each simulation scenario. As is illustrated in the table below we will have over 84% power for a variety of distributional assumptions

Distribution	Uganda parameters	MN parameters	Power
Normal	μ=7.89, σ=1	μ=8.89, σ=1	0.871
Gamma	k=62.25, θ=0.13	k=79.03, θ=0.11	0.874
T + shift	ν= 20, shift=7.89	ν= 20, shift= 8.89	0.841

9.3 POPULATIONS FOR ANALYSES

For comparisons involving the primary endpoint, YFV titers, we will only include study participants who received the YF vaccine. For analysis of secondary endpoints, we will use data from all available study participants.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Medians and interquartile ranges will be presented for descriptions of continuous data. Percentages will be presented for categorical data.

Counts of cell frequency and QIA endpoints involving the percent area will be log-transformed and linear regression analyses will be conducted where appropriate. If there are no detectable cells or there is no staining detected for a large portion of study participants logistic regression modeling will be conducted, instead, with presence or absence of the measure being the outcome.

Two-sided tests will be conducted for all comparisons. Subjects specific random effects will be included in all mixed effects models and permutation p-values will be presented. To control for multiple comparisons, a cut-off of alpha=0.01 will be used for determining statistical significance of all secondary endpoints.

Measures which have non-zero coefficients in lasso models will be considered to be associated with the outcome. Measures which have non-zero correlation coefficients after applying sparse canonical correlation analysis will be considered to be correlated with the outcome measure.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

Mann-Whitney U tests will be used to test for differences in peak YFV titer between the US and Uganda. Analyses will be stratified by gender to investigate if gender has a major impact on the observed differences. No imputation will be conducted if there is missing data.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

Differences in rate of YFV antibody titer decay will be compared between vaccinated Ugandans and Minnesotans using linear mixed effects regression model with measurement time, country, and the interaction between measurement time and country as predictors. Baseline LN measures (area with collagen, area with desmin staining, frequency and size of B cell follicles, and frequency of Tfh cells) will be compared between the two countries using Mann-Whitney U tests.

We will examine the association between vaccine response over time and baseline LN measures using linear mixed effects models adjusting for measurement time and gender.

To examine differences in infection patterns between the two countries, we will perform principal component analyses on the baseline pathseq data. We will test for differences in infection patterns between Uganda and the US populations using logistic regression with country as the outcome and the scores for each of the first five principal components as predictors. Differences in helminth infections between Ugandans and Minnesotans will be compared using Fisher's exact test.

We will examine the association between infection history and baseline LN architecture by fitting separate linear regression models for each measure of baseline LN architecture with gender and the 5 pathseq principal component scores as predictors. The association between helminth infections and baseline LN architecture measures will be assessed using Mann-Whitney U tests.

To elucidate the role of previous infections on inflammation and activation we will use sparse canonical correlation analysis (Reference: Witten, D., Tibshirani, R., & Hastie, T. (2009). A penalized matrix decomposition, with applications to sparse principal components and canonical correlation analysis. Biostatistics., 10(3), 515-534.) to identify sets of organismal sequences correlated with baseline measures of systemic and lymphatic inflammation (plasma cytokines, frequency of TGFß+ and TNF+ cells in lymphoid tissues) and immune activation (Ki67+ and CD69 + cells). The association between helminth infection and measures of inflammation and activation be assessed using a logistic lasso regression model. Measures which have non-zero coefficients in the model will be considered associated with helminth infection.

The association between change in measures of CD8 function (IFNγ, TNFα, and IL-2) after vaccination and measures of LN architecture will be examined by fitting mixed effects linear models using area with collagen staining, area with desmin staining, and measurement time and gender as predictors.

The association between immune function after vaccination and measures of inflammation and activation will be assessed using separate lasso regression for each measure of immune function (antigen specific CD4, CD8 function measures, and CD4 and CD8 subpopulations). Inflammation and activation measures which have non-zero coefficients in the model will be considered predictive of immune function.

We will examine the impact of measures of immune function on YFV response using separate mixed effects linear models with gender, measurement time and measures of immune function (antigen specific CD4, CD8 functional variables, or CD4 and CD8 subpopulations) as predictors. Similar models will be fit for YF specific IgM, IgG1, IgG2, IgG3 and IgG4 responses.

The impact of vaccination on measures of inflammation and activation be assessed using mixed effects repeated measures model with gender, measurement time, and vaccination status as predictors. To assess the impact of vaccination on CD4 and CD8 cells in LT similar models will be fit, but only gender and vaccination status (measurement taken before or after vaccination) will be used as predictors.

Missing data will not be imputed and a significance level of 0.01 will be used for secondary analyses due to the large number of hypotheses to be tested. The results from all of these investigations will be reported so that the reader can assess the effect of multiple hypothesis testing.

9.4.4 SAFETY ANALYSES

Any AEs will be reported annually in a study report that will document AEs and completeness of data. This report will include the total number of AEs for each cohort and the total number of participants in each cohort that experience an AE. AEs will be summarized in terms of severity and relatedness and the number of participants experiencing SAEs will also be reported for each cohort. A line listing will also be included in this report.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Demographic baseline characteristics will be compared between the MN and Ugandan populations using Mann-Whitney U tests (continuous outcomes) or Fisher's exact test (categorical outcomes).

9.4.6 PLANNED INTERIM ANALYSES

N/A- There is no planned interim analysis.

9.4.7 SUB-GROUP ANALYSES

In addition to including gender, we will assess how additional clinical factors (such as age and other demographic variables) influence immune response to vaccination by including relevant clinical factors in to our models.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Individual participant data will not be listed by measure and time point.

9.4.9 EXPLORATORY ANALYSES

The association between change in the CD4 and CD8 T cell populations after vaccination and measures of LN architecture will be examined by fitting mixed effects linear models estimating T cells populations using area with collagen staining, area with desmin staining, and measurement time and gender as predictors.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

A signed consent form will be obtained from the participant. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participant, and this fact will be documented in the participant's record.

Research staff will ensure that candidates understand all elements of the consent form by addressing questions posed during the consent procedure and by asking for verbal confirmation that the candidate has no additional questions and verbally understands the purpose of the study, study intervention, basic procedures, risks, and that participation is voluntary. Participants will be given as much time as they need to read and understand the consent form.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements

- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the University of Minnesota. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by University of Minnesota research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the University of Minnesota.

Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator
Timothy W Schacker, MD
University of Minnesota

MMC 250 516 Delaware Street Minneapolis, MN 55455
Phone: 612-624-9955
E-Mail: *schacker@umn.edu*

10.1.5 SAFETY OVERSIGHT

This study will use local Independent Safety Monitor (ISM) for JCRC and UMN. The ISMs will be independent physicians with relevant expertise and free of conflicts of interest whose primary responsibility will be to provide independent monitoring of the study. Participation will be for the duration of the study. The role will include ongoing review for clinical data for all participants, and also the following items:

1. Determine frequency of safety review needed throughout the study (individually, if applicable)
2. Give input on the protocol and procedures

This role requires both ISMs to review all adverse events and thoroughly investigate any serious and unexpected events. Both ISMs will be able to readily access participant records in real time. The study team shall notify both ISMs via email of any SAE's immediately after becoming aware of any such event. The ISMs and study team will review the case as soon as possible, and where appropriate examining the participant in question. The ISMs will produce a short report on each SAE.

Every year the study team will provide both ISMs a summary report of all the adverse events. The ISMs will produce a short report on your review of these events.

After the first five participants are enrolled, the study team will give both ISM a report of all the adverse events and participant data. The ISMs will review the data for any trends or events that may be clinically significant, and provide a short one page summary report. If no clinically significant items are found after initial review, the study team will then continue to send data to review every one year. If the ISMs' summary report finds items of clinical significance, the frequency of the medical review will be altered as the ISMs see fit.

10.1.6 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

- The CTSI clinical trial monitors will conduct on-site monitoring at the University of Minnesota and virtual monitoring for JCRC. The type of monitoring (e.g., on-site, centralized), frequency (e.g., early, for initial assessment and training versus throughout the study), and extent (e.g., comprehensive (100% data verification) versus targeted or random review of certain data (less

than 100% data verification or targeted data verification of endpoint, safety and other key data variables)), and the distribution of monitoring reports.
- Per Uganda National Counil for Science and Technology, the JCRC clinical trial monitor will monitor all of the JCRC informed consents.
- Clinical trial monitoring will also be conducted by the Ugandan Regulatory Authority.

10.1.7 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion by clinical trial monitors.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements.

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.8 DATA HANDLING AND RECORD KEEPING

10.1.8.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents. Hardcopies of study materials will be kept behind lock and key and only accessible to authorized personnel.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into REDCap, a 21 CFR Part 11-compliant data capture system provided by the University of Minnesota. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents. The data center at University of Minnesota has QA procedures in place that are easily integrated into REDCap. These systems can operate on de-identified data and include functionality that goes beyond what can be accomplished using REDCap, such as data quality checks that involve data from multiple CRFs.

REDCap uses a MySQL database via a secure web interface with data checks used during data entry to ensure data quality. REDCap includes a complete suite of features to support HIPAA compliance, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL database and the web server will both be housed on secure servers. Access to the study's data in REDCap will be restricted to the members of the study team by username and password.

10.1.8.2 STUDY RECORDS RETENTION

Per NIH requirements, study documents should be retained for a minimum of 3 years *from the date of Federal Financial Report (FFR) submission*. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.
HIPAA authorizations are to be retained for 6 years from the date of its creation or the date when it was last in effect, whichever is later.
All study documents at JCRC in Uganda will be stored for a minimum of 5 years per Uganda National Council for Science and Technology (UNCST) and National Drug Authority (NDA) guidelines.

10.1.9 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

10.1.10 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

In addition, this study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.

10.1.11 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Institute of Allergy and Infectious Diseases has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
BX	Biopsy
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
FRCn	Fibroblastic reticular cell network
GALT	Gastric Associated Lymphatic Tissue
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies

HIPAA	Health Insurance Portability and Accountability Act
IA	Immune Activation
IB	Investigator's Brochure
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IHC	Immunohistochemistry
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LN	Lymph Node
LT	Lymphoid Tissue
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PBMCs	Peripheral Blood Monocytes
PI	Principal Investigator
PRA	Plaque Reduction Assay
QA	Quality Assurance
QC	Quality Control
QIA	Quantitative Image Analysis
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
Tfh	T Follicular Helper
TZ	T cell Zone
UP	Unanticipated Problem
US	United States
YF	Yellow Fever
YFV	Yellow Fever Vaccine

11 REFERENCES

1. Fine PE. Variation in protection by BCG: implications of and for heterologous immunity. Lancet. 1995;346(8986):1339-45. Epub 1995/11/18. PubMed PMID: 7475776.
2. Patel M, Glass RI, Jiang B, Santosham M, Lopman B, Parashar U. A systematic review of anti-rotavirus serum IgA antibody titer as a potential correlate of rotavirus vaccine efficacy. J Infect Dis. 2013;208(2):284-94. Epub 2013/04/19. doi: 10.1093/infdis/jit166. PubMed PMID: 23596320.
3. Hanlon P, Hanlon L, Marsh V, Byass P, Shenton F, Hassan-King M, Jobe O, Sillah H, Hayes R, M'Boge BH, et al. Trial of an attenuated bovine rotavirus vaccine (RIT 4237) in Gambian infants. Lancet. 1987;1(8546):1342-5. Epub 1987/06/13. PubMed PMID: 2884455.
4. Lanata CF, Midthun K, Black RE, Butron B, Huapaya A, Penny ME, Ventura G, Gil A, Jett-Goheen M, Davidson BL. Safety, immunogenicity, and protective efficacy of one and three doses of the tetravalent rhesus rotavirus vaccine in infants in Lima, Peru. J Infect Dis. 1996;174(2):268-75. Epub 1996/08/01. PubMed PMID: 8699054.
5. Hallander HO, Paniagua M, Espinoza F, Askelof P, Corrales E, Ringman M, Storsaeter J. Calibrated serological techniques demonstrate significant different serum response rates to an oral killed cholera vaccine between Swedish and Nicaraguan children. Vaccine. 2002;21(1-2):138-45. Epub 2002/11/22. PubMed PMID: 12443672.
6. John TJ. Problems with oral poliovaccine in India. Indian-pediatrics. 1972;9(5):252-6. Epub 1972/05/01. PubMed PMID: 4649422.
7. Fleming DT, McQuillan GM, Johnson RE, Nahmias AJ, Aral SO, Lee FK. Herpes simplex virus type 2 in the United States, 1976 to 1994. NEJM. 1997;337(16):1105-11.
8. John TJ, Christopher S. Oral polio vaccination of children in the tropics. III. Intercurrent enterovirus infections, vaccine virus take and antibody response. American Journal Of Epidemiology. 1975;102(5):422-8. Epub 1975/11/01. PubMed PMID: 1200026.
9. Levine MM. Immunogenicity and efficacy of oral vaccines in developing countries: lessons from a live cholera vaccine. BMC Biol. 2010;8:129. Epub 2010/10/06. doi: 10.1186/1741-7007-8-129. PubMed PMID: 20920375; PMCID: PMC2958895.
10. Patriarca PA, Wright PF, John TJ. Factors affecting the immunogenicity of oral poliovirus vaccine in developing countries: review. Reviews Of Infectious Diseases. 1991;13(5):926-39. Epub 1991/09/01. PubMed PMID: 1660184.
11. Paul Y. Why polio has not been eradicated in India despite many remedial interventions? Vaccine. 2009;27(28):3700-3. Epub 2009/05/26. doi: 10.1016/j.vaccine.2009.03.078. PubMed PMID: 19464552.
12. Muyanja E, Ssemaganda A, Ngauv P, Cubas R, Perrin H, Srinivasan D, Canderan G, Lawson B, Kopycinski J, Graham AS, Rowe DK, Smith MJ, Isern S, Michael S, Silvestri G, Vanderford TH, Castro E, Pantaleo G, Singer J, Gillmour J, Kiwanuka N, Nanvubya A, Schmidt C, Birungi J, Cox J, Haddad EK, Kaleebu P, Fast P, Sekaly RP, Trautmann L, Gaucher D. Immune activation alters cellular and humoral responses to yellow fever 17D vaccine. J Clin Invest. 2014;124(7):3147-58. Epub 2014/06/10. doi: 10.1172/JCI75429. PubMed PMID: 24911151; PMCID: PMC4071376.
13. Estes JD, Li Q, Reynolds MR, Wietgrefe S, Duan L, Schacker T, Picker LJ, Watkins DI, Lifson JD, Reilly C, Carlis J, Haase AT. Premature induction of an immunosuppressive regulatory T cell response during acute simian immunodeficiency virus infection. J Infect Dis. 2006;193(5):703-12. Epub 2006/02/03. doi: JID35261 [pii] 10.1086/500368. PubMed PMID: 16453267.
14. Schacker TW, Nguyen PL, Beilman GJ, Wolinsky S, Larson M, Reilly C, Haase AT. Collagen deposition in HIV-1 infected lymphatic tissues and T cell homeostasis. J Clin Invest. 2002;110(8):1133-9. PubMed PMID: 12393849.
15. Zeng M, Smith AJ, Wietgrefe SW, Southern PJ, Schacker TW, Reilly CS, Estes JD, Burton GF, Silvestri G, Lifson JD, Carlis JV, Haase AT. Cumulative mechanisms of lymphoid tissue fibrosis and T cell depletion in HIV-1 and SIV infections. J Clin Invest. 2011;121(3):998-1008. Epub 2011/03/12. doi: 45157 [pii] 10.1172/JCI45157. PubMed PMID: 21393864; PMCID: 3049394.
16. Zeng M, Southern PJ, Reilly CS, Beilman GJ, Chipman JG, Schacker TW, Haase AT. Lymphoid tissue damage in HIV-1 infection depletes naive T cells and limits T cell reconstitution after antiretroviral therapy. PLoS pathogens.

2012;8(1):e1002437. Epub 2012/01/14. doi: 10.1371/journal.ppat.1002437. PubMed PMID: 22241988; PMCID: 3252371.
17. Lugada ES, Mermin J, Kaharuza F, Ulvestad E, Were W, Langeland N, Asjo B, Malamba S, Downing R. Population-based hematologic and immunologic reference values for a healthy Ugandan population. Clin Diagn Lab Immunol. 2004;11(1):29-34. Epub 2004/01/13. PubMed PMID: 14715541.
18. Messele T, Abdulkadir M, Fontanet AL, Petros B, Hamann D, Koot M, Roos MT, Schellekens PT, Miedema F, Rinke de Wit TF. Reduced naive and increased activated CD4 and CD8 cells in healthy adult Ethiopians compared with their Dutch counterparts. Clinical And Experimental Immunology. 1999;115(3):443-50. Epub 1999/04/08. PubMed PMID: 10193416.
19. Malaza A, Mossong J, Barnighausen T, Viljoen J, Newell ML. Population-based CD4 counts in a rural area in South Africa with high HIV prevalence and high antiretroviral treatment coverage. PLoS One. 2013;8(7):e70126. Epub 2013/07/31. doi: 10.1371/journal.pone.0070126. PubMed PMID: 23894603; PMCID: PMC3720940.
20. Tsegaye A, Messele T, Tilahun T, Hailu E, Sahlu T, Doorly R, Fontanet AL, Rinke de Wit TF. Immunohematological reference ranges for adult Ethiopians. Clin Diagn Lab Immunol. 1999;6(3):410-4. Epub 1999/05/04. PubMed PMID: 10225845.
21. Valiathan R, Deeb K, Diamante M, Ashman M, Sachdeva N, Asthana D. Reference ranges of lymphocyte subsets in healthy adults and adolescents with special mention of T cell maturation subsets in adults of South Florida. Immunobiology. 2014;219(7):487-96. Epub 2014/03/26. doi: 10.1016/j.imbio.2014.02.010. PubMed PMID: 24661720.
22. Cremasco V, Woodruff MC, Onder L, Cupovic J, Nieves-Bonilla JM, Schildberg FA, Chang J, Cremasco F, Harvey CJ, Wucherpfennig K, Ludewig B, Carroll MC, Turley SJ. B cell homeostasis and follicle confines are governed by fibroblastic reticular cells. Nat Immunol. 2014;15(10):973-81. Epub 2014/08/26. doi: 10.1038/ni.2965. PubMed PMID: 25151489; PMCID: PMC4205585.
23. Schacker TW, Brenchley JM, Beilman GJ, Reilly C, Pambuccian SE, Taylor J, Skarda D, Larson M, Douek DC, Haase AT. Lymphatic tissue fibrosis is associated with reduced numbers of naive CD4+ T cells in human immunodeficiency virus type 1 infection. Clin Vaccine Immunol. 2006;13(5):556-60. Epub 2006/05/10. doi: 13/5/556 [pii]
10.1128/CVI.13.5.556-560.2006. PubMed PMID: 16682476.
24. Kelsoe G. The germinal center: a crucible for lymphocyte selection. Seminars-in-immunology. 1996;8(3):179-84. Epub 1996/06/01. doi: 10.1006/smim.1996.0022. PubMed PMID: 8738917.
25. Allen CD, Okada T, Cyster JG. Germinal-center organization and cellular dynamics. Immunity. 2007;27(2):190-202. Epub 2007/08/29. doi: 10.1016/j.immuni.2007.07.009. PubMed PMID: 17723214; PMCID: PMC2242846.
26. Akondy RS, Monson ND, Miller JD, Edupuganti S, Teuwen D, Wu H, Quyyumi F, Garg S, Altman JD, Del Rio C, Keyserling HL, Ploss A, Rice CM, Orenstein WA, Mulligan MJ, Ahmed R. The yellow fever virus vaccine induces a broad and polyfunctional human memory CD8+ T cell response. J Immunol. 2009;183(12):7919-30. Epub 2009/11/26. doi: 10.4049/jimmunol.0803903. PubMed PMID: 19933869; PMCID: PMC3374958.
27. Crotty S. T follicular helper cell differentiation, function, and roles in disease. Immunity. 2014;41(4):529-42. Epub 2014/11/05. doi: 10.1016/j.immuni.2014.10.004. PubMed PMID: 25367570; PMCID: PMC4223692.
28. Crotty S. Follicular helper CD4 T cells (TFH). Annual review of immunology. 2011;29:621-63. Epub 2011/02/15. doi: 10.1146/annurev-immunol-031210-101400. PubMed PMID: 21314428.
29. Kim CH, Lim HW, Kim JR, Rott L, Hillsamer P, Butcher EC. Unique gene expression program of human germinal center T helper cells. Blood. 2004;104(7):1952-60. Epub 2004/06/24. doi: 10.1182/blood-2004-03-1206. PubMed PMID: 15213097.
30. Kim JR, Lim HW, Kang SG, Hillsamer P, Kim CH. Human CD57+ germinal center-T cells are the major helpers for GC-B cells and induce class switch recombination. BMC Immunol. 2005;6:3. Epub 2005/02/08. doi: 10.1186/1471-2172-6-3. PubMed PMID: 15694005; PMCID: PMC548684.
31. Kaleebu P, J L, Nanvubya A, F K, W J, P P, M P, A N, P K, G TP, J S, M Y, P F, B M, D M, G T, M R, C L, R W, R S, W K, N K, J G, H K, D V, K C, F P, S D, T H, G P. Abstracts of the Eighth EDCTP Forum, 6-9 November 2016. BMJ Glob Health. 2017;2(Suppl 2):A1-A72. Epub 2017/06/08. doi: 10.1136/bmjgh-2016-000260. PubMed PMID: 28589036; PMCID: PMC5418676.