Section	Version 2.0 changes
Clinical Protocol	Replace Version 1.0 with Version 3.0 Update protocol date

CLINICAL PROTOCOL

PROTOCOL TITLE: A phase I/II study of the immunogenicity of the yellow fever vaccine 17D (YFVax®) in adults with prior 17D vaccination.
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1 SYNOPSIS

Title of the Protocol: A phase I/II study of the immunogenicity of the yellow fever vaccine 17D (YFVax®) in adults with prior 17D vaccination.

Overview: Yellow fever virus (YFV) is the prototype flavivirus, closely related to dengue (DENV), Zika (ZIKV) and Japanese encephalitis (JEV) viruses. YFV is transmitted primarily by Aedes aegypti mosquitoes in urban transmission cycles and by Haemogogus spp. forest canopy mosquitoes in sylvatic, non-human primate transmission cycles. Historically YFV has been the most important arthropod-borne viral pathogen of humans worldwide, causing explosive epidemics in Europe, the Americas and Africa in the 18th, 19th and early 20th centuries, and it remains endemic to at least 34 African and 13 South American countries. Control of YFV has been accomplished largely through vector abatement and mass vaccination with the YFV vaccine 17D. In the early 2000's the WHO estimated that there were approximately 200,000 cases/year, with 30,000 deaths, but in the past decade falling vaccination rates and spillover of sylvatic YFV have led to a dramatic resurgence of YFV disease, with an estimated 130,000 severe cases and 78,000 fatalities in Africa in 2013, an estimated 7,334 sylvatic-urban cases in Angola and the Democratic Republic of Congo in 2015-2016, and a series of sylvatic outbreaks in Brazil since 2016 that threaten to spill into urban settings.
Since its development by Max Theiler in 1937, the live-attenuated virus (LAV) YFV vaccine 17D—administered via population-wide vaccination—has been the cornerstone of YFV control. Despite being widely regarded as highly immunogenic and durable, historically the vaccine has been administered as a prime followed by boosting every 10 years. However, the rise in global demand for vaccine doses because of recent YFV outbreaks has resulted in unprecedented worldwide shortages of 17D. These critical shortages have led to strategies to extend vaccine stocks, including a directive by the CDC and WHO to replace the long-standing 10-year boost with once-in-a-lifetime vaccination for most vaccinees. The policy changes should relieve short-term pressure on the vaccine supply, but they move 17D vaccination policies into uncharted waters. Scrutiny and criticism of these strategies, particularly that of dropping the 10-year boost, make clear that definitive studies to validate or revise these strategies have not been done. Specifically, the immune correlates of protection, how the vaccine induces protective immunity, and who maintains and who loses long-term protection have not been well-defined.

Rationale: In 1973 Mason et al demonstrated a clear correlation between 17D induced neutralizing antibody titers and protection against lethal challenge with the virulent YFV Asibi strain in Rhesus macaques. Subsequently, neutralizing antibody titers have become the most widely accepted correlate of 17D-induced protection and are believed to be necessary and sufficient for YFV immunity. Generalizability of Mason's data is difficult, however, because Mason's protective threshold titer was demonstrated in hon-human primates and based on a ratio between log10 pre- and post-vaccination neutralizing antibody titers – the log neutralizing index (LNI) –equivalent LNI or alternate protective neutralizing antibody titer has never been validated in humans. Nevertheless, 90% percent plaque reduction neutralization test (PRNT90 or NT90) titers >1:10 are often taken as an immune correlate of protection, despite a lack of supporting data. A limited number of studies using 17D booster doses as a live-attenuated challenge model (boosting is a true homologous live-virus challenge) have evaluated neutralized antibody titers that confer "sterilizing" immunity. In these studies, sterilizing immunity is defined as a less than four-fold rise in neutralizing antibody titer post-boost and/or no detection of vaccine virus by virus isolation or PCR. These studies suggest that NT80 or NT90 titers >1:40 are probably required for reliable sterilizing immunity against live attenuated 17D challenge. Drawing upon the model that 17D boost is a homologous live-virus challenge, we will prospectively recruit and boost 35 individuals with history of 1 prior 17D vaccination ~10 years

prior. We will characterize pre- and post-boost neutralizing antibody titers, boost vaccine viral load, and innate and B-cell responses in boosted vaccinees. We will identify titers below which vaccine viral load and/or antibody boost responses are detected and higher titers that confer sterilizing immunity without detectable viral load or antibody titer boost, establishing both stringent and relaxed correlates of antibody protection. We will also assess whether some vaccinees with undetectable neutralizing antibodies are still protected against 17D challenge, setting the stage to explore the extent to which protection may be provided by other immune mechanisms.

Study Design: This is a prospective single arm study of the immunogenicity of the yellow fever vaccine 17D (YFVax®, Sanofi Pasteur, Swiftwater, PA) in adults who have documented history of prior yellow fever vaccination 8 or more years prior. Study subjects will receive a single YFV-17D vaccination and be followed for 30 days after vaccination with blood samples collected on days 1, 2, 4, 6, 8, 10, 12, 14 and 28 post vaccination. The study is designed to enroll 34 vaccinees. Study duration is expected to be no longer than 60 days. Subject accrual will be completed in 2 years.

Objectives:
Primary Objective: To assess 17D vaccine viremia and antibody titer boost post vaccination.
Secondary Objectives: To assess the magnitude and specific innate immune responses to vaccination, to asses pre- and post-vaccination B and T-cell populations and responses to vaccination.
Safety evaluations: Diary cards will record solicited adverse events (AEs) for local reactions for 7 days following vaccination and solicited systemic AEs for 14 days post vaccination. Unsolicited AEs will be monitored for 28 days after vaccination.

Study Population:
Inclusion Criteria:
Male or female subjects who meet all of the following criteria are eligible for enrollment:
- Are 20 to 49 years of age at the time of enrollment
- Have a documented history of a single yellow fever vaccination with YFVax.
- Not previously vaccinated for tick-borne encephalitis virus (TBEV), Japanese encephalitis virus (JEV), or dengue virus.
- Subjects of childbearing potential who are willing to use an acceptable form of contraception 30 days prior to the YFV-17D vaccination, and until their participation in this study is completed, and who have a negative pregnancy test and express the intent not to conceive a child while participating in the study. If the female subject is sexually active and is of childbearing potential, she must agree to use an acceptable method of birth control (birth control pills, Depo-Provera, Norplant, intrauterine device [IUD], NuvaRing, or diaphragm/condom plus spermicide) or remain abstinent.

Exclusion Criteria:
Subjects who meet any of these criteria are not eligible for enrollment:
- A known history of infection or serologic evidence consistent with prior infection with YFV, dengue fever, JEV, or West Nile Virus (WNV).
- Have a history of acute hypersensitivity reaction to any components of the yellow fever vaccine (including egg or gelatin).

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- any history of progressive or severe neurologic disorder, seizure disorder or neuro-inflammatory disease (eg, Guillain-Barré syndrome).
- known or suspected impairment/alteration of immune function.
- Known or suspected liver dysfunction.
- serious chronic or progressive disease according to the judgment of the investigator (eg, neoplasm, hematologic malignancies, diabetes; cardiac, renal, or hepatic disease).
- a body mass index (BMI) greater than or equal to 35 kg/m2 (=weight in kg/ height in meters2).
- women who are pregnant or breastfeeding.
- Subjects of childbearing potential, sexually active with men, and have not used any of the acceptable contraceptive methods for at least 2 months prior to vaccination. Subjects who fail this screen may initiate or continue acceptable contraception until 2 months of use and then rescreen.

Clinical and Laboratory Evaluations: Study subjects will undergo a physical exam prior to vaccination and on post-vaccination days 6 and 14. Complete blood count and complete metabolic panel tests will be drawn and reviewed prior to vaccination. Vaccination will not proceed if CBC, creatine, or liver function tests fall outside the range of normal. Liver function tests will be repeated on day 6, day 14, and day 28. Serum will be tested on days 1, 2, 4, 6, 8, 10, 12, and 14 for vaccine viremia using plaque assay, virus isolation by tissue culture and virus genomic copy by quantitative reverse transcription polymerase chain reaction (qRT-PCR). Serum will be tested for innate immune response markers will be assayed on days 1, 2, 8, 14 and 28.

Statistical considerations and power analyses: To execute this aim, we will work with Dr. Naleway at KPNW to identify previously vaccinated subjects 8 or more years post vaccination. KNPNW vaccinates ~400 individuals/year aged 20-49 years old. Assuming we contact 400 subjects/year with ~4% uptake, we expect to recruit seventeen subjects/year for 2 years for a total of 34 subjects over the study period. Subjects will be recruited pre-vaccination and followed for 30 days post-vaccination with blood draws identical to those through Day 30 in Aim 1. We assume little to no loss-to-follow up as this study will be conducted over only 60 days, at the end of study Year 5 we expect we will have enrolled at least 32 subjects over 2 years to fully evaluate.
Power calculations. Based on prior studies, we assume a priori a sterilizing threshold of NT90 >1:40. Assuming that 10% of 16 subjects with titers >1:40 boost, and 75% of 16 subjects with titers <1:40 boost, against the null that 50% will boost each in group, the study has power=0.85 ($\chi 2$=12.28, DF=3, α=0.05, 32 subjects) to detect a difference in boost between two groups divided at an NT90 threshold of >1:40. The study retains power >0.80 with as few as 22 subjects under the same assumptions, and 20 subjects will have power=0.76. There are no prior data available to estimate the proportions of subjects who will develop a detectable viral load on 17D re-vaccination, and we consider evaluation for viral load exploratory but extremely important for the field. We assume that viral load is most likely to be detected in re-vaccinated subjects with NT90<1:40, or 16 (50%) subjects, and we contend that the null hypothesis is that no more than 1 in 100 will have a detectable viral load as this is consistent with reasoning behind CDC and WHO recommendations for once-in-a-lifetime vaccination. Under these

assumptions, the study has power=0.80 (test of 1 proportion1) to detect a viral load in at least 1 in 10 subjects.

Human Subjects:
Vaccine Risks. YFV-17D is Food and Drug Administration (FDA)-approved in the US for both primary vaccination in persons aged >6 months traveling to YFV endemic regions and booster vaccination in subjects considered high risk for YFV exposure. YFV-17D is widely
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17D is acknowledged as one of the safest live attenuated vaccines in use. Over 500 million people have been immunized with 17D. YFV-17D has a strong record of safety and tolerability dating back to its introduction in 1936. Common reactions to 17D vaccination include mild headaches, myalgia, low-grade fevers, or other minor symptoms for 5-10 days. Other symptoms may include localized injection site pain, swelling, erythema, or warmth for up to a week after vaccination. In one large phase III trial,2 the most common systemic side effects were headache (33% of subjects), myalgia (25%), malaise (19%), fever (15%), and chills (11%). The most serious and potentially life-threatening AEs related to this vaccine are neurotropic (YEL-AND) and viscerotropic disease (YEL-AVD). In the US civilian sector analyzed from 2000 to 2006, there were 1.5 million doses of YFV-17D administered. YFV-17D-associated neurotropic disease (YEL-AND) has an approximate incidence of 0.8 cases per 100,000 vaccinations (all ages) but is not typically lethal. YFV-17D-associated viscerotropic disease (YEL-AVD) has an incidence of 0.4 cases per 100,000 vaccinations (all ages) with an approximate 50% mortality rate, resulting in up to 2 fatalities per million vaccinations. There is a reduced incidence of approximately 0.07 to 0.24 cases per 100,000 vaccinations in subjects aged 19 to 49.

Risk in booster vaccination: The CDC reports data from observational studies that include 333 million vaccine doses, with 1,255 SAEs overall; vaccination type (primary vs boost) was reported for 201 SAEs, with 14 (7%) of reported SAEs occurring on booster vaccination. CDC reports YEL-AND observational data for 462 million vaccine doses distributed with 218 YEL-AND cases: vaccination type was known for 110 of the YEL-AND cases, of which 3 (3%) were booster vaccinations. CDC also reports YEL-AVD observational data for 437 million vaccine doses distributed with 72 YEL-AVD cases; vaccination type was known for 31 of the YEL-AVD cases, of which 1 (3%) was a booster vaccination. This study has safety lab provisions for early detection and management of YEL-AVD.

Blood Draw Risks: Risks associated with drawing blood from the arm include some pain when the needle is inserted and a small risk of bruising and/or infection at the place where the needle enters the arm. Some people may experience lightheadedness, nausea, and/or fainting.

Benefits. The proposed studies are not expected to be of direct benefit to study subjects. However, their participation in these studies may further increase our understanding of the mechanisms underlying long term immunity following 17D vaccination.

2 Summary of study events

Visit	Screening visit	Baseline V1	V2	V3	V4	V5	V6	V7	V8	V9
Visit window (days post vaccination)	up to 30 days pre-vaccination	0	2 (±1)	4 (±1)	6 (±1)	8 (±1)	10 (±1)	12 (±1)	14 (±1)	28 (±7)
Informed consent	X									
Assessment of eligibility criteria	X									
Demographics, medical history, and prior medication/ vaccination	X									
Concomitant medications/vaccinations during study period	X									
Travel History Questionnaire	X									
Urine Pregnancy Test (if applicable)	X									
Blood sample for plasma and peripheral blood mononuclear cells		20 mL			10 mL				10 mL	10 mL
Blood sample for serum viremia and/or innate immune response as specified in the protocol[2]		3 mL	3 mL	3 mL	3 mL	3 mL	3 mL	3 mL	3 mL	
Blood sample for serology (screening and post-vaccination antibodies)	5.0 mL									5.0 mL
Blood sample for CBC	3.0 mL									
Blood sample for CMP	4.0 mL									
Blood sample for Paxgene[4]		2.5 mL								
Total Blood Volume	12.0 mL	25.5 mL	3 mL	3 mL	13 mL	3 mL	3 mL	3 mL	13 mL	15 mL
Study Diary		X	X	X	X	X	X	X	X	X
YFV Vaccination		X								
Full Investigator examination	X	X								X
Focused investigator examination as needed			X	X	X	X	X	X	X	

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4 ABBREVIATIONS

ACIP	Advisory Committee on Immunization Practices
AE	Adverse Event
AUC	Area Under the Curve
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	Confidence Interval
CPT	Cell Preparation Tube
CO2	Carbon Dioxide
CTL	Cytotoxic T-Lymphocyte
EDC	Electronic Data Capture
ELISA	Enzyme-Linked Immunosorbent Assay
FACS	Fluorescence-Activated Cell Sorting
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HEP	Hepatitis
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
ID	Identification
Ig	Immunoglobulin
IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IUD	Intrauterine Device
IVIG	Intravenous Immunoglobulin
JEV	Japanese Encephalitis Virus
LNI	Log10 Neutralization Index
MedDRA	Medical Dictionary for Regulatory Activities
NIH	National Institutes of Health
NIAID	National Institute of Allergy and Infectious Diseases
OHSU	Oregon Health and Science University
PRNT	Plaque Reduction Neutralization Test
PBMC	Peripheral Blood Mononuclear Cell
PFU	Plaque Forming Unit(s)
PI	Principal Investigator
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
SAE	Serious Adverse Event
SC	Subcutaneous(ly)
SD	Standard Deviation
SDCC	Statistical and Data Coordinating Center
SSL	Secure Sockets Layer
TBEV	Tick-Borne Encephalitis Virus
US	United States (of America)
VAERS	Vaccine Adverse Event Reporting System
WNV	West Nile Virus
YEL-AND	Yellow Fever Vaccine-Associated Neurotropic Disease Y
EL-AVD	Yellow Fever Vaccine-Associated Viscerotropic Disease

YF Yellow Fever
YFV Yellow Fever Virus
YFV-17D Yellow Fever Vaccine Virus from Sanofi Pasteur (YF-VAX®)
YF-VAX® Yellow Fever Vaccine Manufactured by Sanofi Pasteur

5 BACKGROUND AND RATIONALE

5.1 Introduction

Yellow fever virus (YFV) is the prototype flavivirus, closely related to dengue (DENV), Zika (ZIKV) and Japanese encephalitis (JEV) viruses. YFV is transmitted primarily by *Aedes aegypti* mosquitoes in urban transmission cycles and by *Haemogogus spp*. forest canopy mosquitoes in sylvatic, non-human primate transmission cycles. Historically YFV has been the most important arthropod-borne viral pathogen of humans worldwide, causing explosive epidemics in Europe, the Americas and Africa in the 18th, 19th and early 20th centuries,[1,2] and it remains endemic to at least 34 African and 13 South American countries. Control of YFV has been accomplished largely through vector abatement and mass vaccination with the YFV vaccine 17D.[2] In the early 2000's the WHO estimated that there were approximately 200,000 cases/year, with 30,000 deaths,[3] but in the past decade falling vaccination rates and spillover of sylvatic YFV have led to a dramatic resurgence of YFV disease, with an estimated 130,000 severe cases and 78,000 fatalities in Africa in 2013,[4] an estimated 7,334 sylvatic-urban cases in Angola and the Democratic Republic of Congo in 2015-2016,[6] and a series of sylvatic outbreaks in Brazil since 2016 that threaten to spill into urban settings.[7,8] Ominously, urban city parks in Sao Paolo were closed in 2017 after at least one park-dwelling monkey died from YFV[9], while the Republic of Congo reported at least once confirmed case of YFV in the heart of the port city of Porte Noire in July, 2018[10]. Additionally, 11 YFV cases were imported by travelers into China from Angola in 2016,[11] potentially exposing the largely unvaccinated Asian population to epidemic transmission. Taken together, the rising incidence of YFV outbreaks in previously well-controlled endemic settings and introductions into unvaccinated populations in endemic and non-endemic[8] settings corroborate the enormous risk YFV still poses to the global population.

Since its development by Max Theiler in 1937, the live-attenuated virus (LAV) YFV vaccine 17D—administered via population-wide vaccination—has been the cornerstone of YFV control. YFV-17D is the parental vaccine strain from which all current YFV vaccines are derived; there are three sub-strains used by global manufacturers, 17D-204 (YF-Vax® in US, Stamaril® in EU), 17D-213 in and 17DD in Latin America,[12] which have been found to be equivalent in trials.[12,13] Despite being widely regarded as highly immunogenic and durable, historically the vaccine has been administered as a prime followed by boosting every 10 years. However, the rise in global demand for vaccine doses because of recent YFV outbreaks has resulted in unprecedented worldwide shortages of 17D.[14] These critical shortages have led to strategies to extend vaccine stocks, including a directive by the CDC and WHO to replace the long-standing 10-year boost with once-in-a-lifetime vaccination for most vaccinees, and global and national health organizations (WHO, Brazilian MOH) exploring fractional rather than full-dose vaccination.[1] The policy changes should relieve short-term pressure on the vaccine supply, but they move 17D vaccination policies into uncharted waters. Scrutiny and criticism of these strategies, particularly that of dropping the 10-year boost, make clear that *definitive studies to validate or revise these strategies have not been done.* Specifically, the immune correlates of protection, how the vaccine induces protective immunity, and who maintains and who loses long-term protection have not been well-defined.

5.2 Rationale

In 1973 Mason *et al*[14] demonstrated a clear correlation between neutralizing antibody titers and protection against lethal challenge with the virulent YFV Asibi strain at 20 weeks post-vaccination with 17D in Rhesus macaques. Subsequently, neutralizing antibody titers have become the most widely accepted correlate of 17D-induced protection and are believed to be necessary and sufficient for YFV immunity.[15] Generalizability of Mason's data is difficult,

however, because Mason's protective threshold titer was based on a ratio between log_{10} pre- and post-vaccination neutralizing antibody titers – the log neutralizing index (LNI) –equivalent LNI or alternate protective neutralizing antibody titer *has never been validated in humans* for symptomatic or lethal wild-type infection. The sporadic, deadly nature of YFV outbreaks, the remote settings in which they often occur, and the logistic challenges of obtaining pre-vaccination titers in vaccinees in endemic areas effectively preclude prospective human studies that could determine an antibody titer that protects against wild-type YFV disease. Nevertheless, 90% percent plaque reduction neutralization test ($PRNT_{90}$ or NT_{90}) titers $\geq$1:10 are often taken as an immune correlate of protection[16], despite a lack of supporting data.

A limited number of studies have used 17D booster doses as a live-attenuated challenge model (boosting is a true homologous live-virus challenge) to evaluate neutralized antibody titers that confer differing degrees of immunity. In these studies, "sterilizing" immunity is defined as a less than four-fold rise in neutralizing antibody titer post-boost and/or no detection of vaccine virus by virus isolation or PCR. These studies suggest that NT_{80} or NT_{90} titers $\geq$1:40 are probably required for reliable sterilizing immunity against live attenuated 17D challenge (Table 1). In 1998 Reinhardt *et al.*[17] boosted 5 subjects $\geq$10 years post first vaccination. All 5 subjects had pre-boost NT_{90} titers ranging from ~1:45 to 1:90 and none had a four-fold or greater boost in NT_{90} post-boost and none had a detectable viral load by PCR. While a threshold-sterilizing NT was not identified, as all five subjects were fully protected, Reinhardt showed *that 17D elicits durable sterilizing immunity correlated with an NT_{90} at of least $\geq$1:45*. In a much larger study, Hepburn *et al*.[18] surveyed 1029 laboratory technicians working with wild-type YFV from 1976-2002 using NT_{80} against the wild-type YFV Asibi strain. When a surveillance NT_{80} dropped <1:40 or a fixed number of years had passed, the lab worker was boosted and had follow-up serology. Among subjects with NT_{80} >1:40, 71/79 (90%) had sterilizing immunity. This proportion dropped to 42/121 (35%) for subjects with NT_{80} between 1:10 and 1:40, and only 183/829 (22%) of subjects with NT_{80} $\leq$1:10 (Table 5.1) had sterilizing immunity. Hepburn *et al.* had two critical findings: 1) there was a clear "dose-response" relationship between pre-boost NT_{80} and loss of sterile immunity, and 2) substantial evidence that YFV immunity wanes over time, with 829/1029 subjects NT_{80} titers vs Asibi falling to <1:10 over the time period studied. Most recently, Kongsgaard *et al.*[19] conducted a prospective study of 28 Danish individuals presenting for 17D boost 9-36 years after primary vaccination. Pre-boost, 23 of the 28 subjects had 50% neutralization titers (NT_{50}) $\geq$1:10 (NT_{90} titers were not reported), while the remaining five had NT_{50} $\leq$1:10. Overall, 21/28 subjects boosted, with 78% (18/23) in the $\geq$1:10 group and 3/5 in the <1:10 group. While Kongsgaard *et al.*[19] did not further stratify by NT_{50} or report individual results, they reported that higher pre-boost NT_{50} titers significantly correlated with less-than-four-fold rises in NT_{50} titers [18]. Importantly, this study *demonstrated that less stringent NT_{50} titers $\geq$1:10 are neither above a threshold of protection nor predictive of sterilizing immunity.*

Study	Threshold	Sterile immunity/total N	% Sterile immunity
Reinhardt *et al.*,	$PRNT_{90}$ >1:40	5/5	100%
Hepburn *et al.*,	$PRNT_{80}$ >1:40	71/79	90%
Hepburn *et al.*, 2002	$PRNT_{80}$ >1:10	42/121	35%
Hepburn *et al.*, 2002	$PRNT_{80}$ <1:10	183/829	22%
Kongsgaard *et al.*,	$PRNT_{50}$ >1:10	5/23	22%
Kongsgaard *et al.*	$PRNT_{50}$<1:10	2/5	40%

Table 5.1. Summary of studies describing proportions of subjects with sterilizing immunity (<4-fold rise between pre- and post-boost in antibody titer and/or no detection of vaccine virus by irus isolation or PCR) on 17D re-vaccination.

The clinical significance of loss of YFV-neutralizing antibodies has become increasingly apparent: while the CDC 2015 recommendations reported only 18 confirmed and documented cases of 17D vaccine failure[38] out of >540 million administered 17D doses, more recent observational data suggest that vaccine failure occurs much more frequently. The Pan American Health Organization (PAHO) has made publicly available confirmed YFV disease cases for Peru, Brazil, Columbia and Bolivia from 2000-2014.[39] Of 1071 cases, 61% (653/1071) had not been vaccinated, 8% (85/1071) had been vaccinated, and an additional 31% (333/1071) had unknown vaccine status. This last proportion is expected to include additional vaccine breakthrough cases. A separate study of sylvatic YFV in Brazil[25] evaluated 831 YFV cases from 1972-2008 reported to the Ministry of Health, finding that 45% (372/831) of reported cases had no recorded vaccine status but the remaining 55% (459/831) had a recorded status, of whom only three had been vaccinated <10 years earlier, but the remaining 456 reportedly were vaccinated $\geq$10 years before infection.[2,25] While limited, these data strongly suggest loss of YFV neutralizing antibodies is clinically important, even lethal, and that the risk increases for some proportion of vaccinees 10 our more years post-vaccination.

Before changing 17D vaccination recommendations from 10-year boost to once-in-a-lifetime vaccination, the CDC reviewed prior research on long- term YFV immunity comprised of 13 cross-sectional serosurveys, finding an average of 88% of vaccinees remain seropositive for YFV-neutralizing antibodies (Table 3), which suggests the 10-year boost was not necessary. However, careful review of this prior research shows substantial heterogeneity in both methods and results, calling into question the overall conclusion, including: 1) the conflation of human antibody titers and mouse protection studies, 2) the conflation of immunity in endemic and non-endemic populations, and 3) treating NT titers with different neutralization cutoffs as comparable[2]. Specifically: four[29-32] of the 13 studies were mouse protection studies *for which no correlation of protection in humans has been reasonably established*[2]; of the remaining human studies, four were conducted in endemic countries[33-36] and five in non-endemic countries [17,26,27,37,38]. When stratified and pooled by endemic/non-endemic studies, 92.7% (359/387) of endemic subjects were seropositive, but only 83.7% (282/337) of non-endemic subjects were seropositive. This disparity is highly statistically significant ($P<0.001$, χ^2 test). This disparity may be due to unreported YFV vaccine boosts in endemic subjects, infection with other flaviviruses in endemic subjects, age differences in vaccinees, or other unknown confounders/effect modifiers. Irrespective, these data argue that endemic and non-endemic populations should not be conflated, with non-endemic studies representing the worst-case scenario for vaccine efficacy. Once segregated by endemic/non-endemic origin and adjusted for differences in neutralizing antibody titer thresholds, we estimate that *at least one in five, ~20%, vaccinees become seronegative by 10 or more years post-vaccination*, while the remainder appear to maintain life-long protective neutralizing antibody titers (Figure 1, Wanjeri *et al.* accepted, Journal of Infectious Diseases). The finding that one in five 17D vaccinees may lose protective neutralizing antibodies by 10 or more years post-vaccination has significant implications for both vaccine deployment and vaccinee safety, but also offers a unique opportunity to compare and contrast 17D immunity over time between seronegative and seropositive subjects and define the determinants of long-term 17D serostatus.

Taken together, we find: 1) substantial evidence that neutralizing antibodies play the critical role in protection against YFV infection following vaccination, 2) at least one in five 17D vaccinees living in non-endemic countries will lose protective neutralizing antibody titers, for which there are, 3) potentially substantial safety and public health implications. However, the studies supporting these preliminary conclusions are limited by their retrospective and cross-sectional design, providing no knowledge of why or when vaccinees become seronegative, or the clinical and epidemiologic implications of seronegative status.

The rationale for this trial is largely established by the Hepburn et al.[18] study that provided substantial data supporting the use of 17D boost as a challenge model for sterilizing immunity. However, Hepburn has several key shortcomings that limit generalizability to other vaccinee populations: 1) it was not specifically designed to test immunity at 10 years post prime, but rather assessed boost when neutralizing antibody titers dropped below a certain level, 2) many subjects had received more than 1 boost, 3) non-standard wild-type Asibi strain was used in the PRNT assay and an 80% rather than 90% neutralization cut-off was reported, and 4) vaccine viremia was not assessed. While measuring boost in neutralization titer has been a standard approach to characterizing strength of protective immunity from 17D vaccination, measuring viral load on boost has been an overlooked but critically important metric for characterizing long-term immunity. The Hepburn study was retrospective and not positioned to assess vaccine viral load in boosted lab workers, but the Kongsgaard study,[18] although done in a prospective manner, did not assess viral load in vaccinees in the acute 1- to 14-day post-vaccination period. Reinhardt et al. did assess viral load, and found none, but the study was quite small and only looked at vaccinees with neutralization titers >1:40, a titer that, based on Hepburn et al., appears to confer sterilizing immunity. A detectable viral load in a boosted vaccinee suggests both the potential for transmissible or symptomatic breakthrough viremia and is the only way to assess whether "undetected" T-cell or low-level neutralizing antibodies have the potential to protect in "seronegative" vaccinees or if these vaccinees with undetectable titers are essentially "naïve."

Drawing upon the model that 17D boost is a homologous live-virus challenge, we will prospectively recruit and boost 35 individuals with history of 1 prior 17D vaccination ~10 years prior. We will characterize pre- and post-boost neutralizing antibody titers, boost vaccine viral load, and innate and B-cell responses in boosted vaccinees. We will identify titers below which vaccine viral load and/or antibody boost responses are detected and higher titers that confer sterilizing immunity without detectable viral load or antibody titer boost, establishing both stringent and relaxed correlates of antibody protection. We will also assess whether some vaccinees with undetectable neutralizing antibodies are still protected against 17D challenge, setting the stage to explore the extent to which protection may be provided by other immune mechanisms.

5.3 Rationale for Selection of Study Population

To minimize study risks, this study will not include any vulnerable populations (*i.e.* prisoners, pregnant women, children, neonates, and/or adults lacking capacity). This study will involve healthy subjects 20 to 49 years of age with documented history of prior yellow fever vaccination. This age range for inclusion was determined based on low susceptibility of young to middle aged adults to SAEs following YFV-17D vaccination (See 5.5) below. YFV-17D is an FDA-approved vaccine but it is contraindicated for vaccination of infants younger than 9 months of age because of an increased risk of encephalitis. It is also contraindicated in immunosuppressed subjects and subjects with known egg hypersensitivity (see YF-VAX® package insert for details). YFV-17D vaccination is generally considered safe for adults, with the most common side effects being flu-like symptoms. However, rare cases of serious or lethal infections stemming from YFV-17D vaccination have been reported. More than 500 million people have been immunized with this vaccine worldwide, with an estimated mortality rate of 0.04 to 2.1 deaths/million doses administered. However, these statistics include individuals of all ages (including the young and elderly who are more susceptible to SAEs) and combine primary and booster vaccinations.

5.4 Safety and Risks of YFV-17D In Humans

17D has a strong record of safety and tolerability dating back to its introduction in 1936. Safety data suggest that booster vaccination as at least 10-fold lower risk of a vaccine related severe adverse event (SAE) compared to primary vaccination. The CDC reports data[74] from observational studies that include 333 million vaccine doses, with 1,255 SAEs overall; vaccination type (primary vs boost) was reported for 201 SAEs, with 14 (7%) of reported SAEs occurring on booster vaccination. The most serious and potentially life-threatening AE related to this vaccine are neurotropic (YEL-AND) and viscerotropic disease (YEL-AVD). CDC reports YEL-AND observational data[74] for 462 million vaccine doses distributed with 218 YEL-AND cases: vaccination type was known for 110 of the YEL-AND cases, of which 3 (3%) were booster vaccinations. CDC also reports YEL-AVD observational data for 437 million vaccine doses distributed with 72 YEL-AVD cases;[74] vaccination type was known for 31 of the YEL-AVD cases, of which 1 (3%) was a booster vaccination. Subjects will be screened for reported adverse reactions at first 17D vaccination and excluded if positive.

5.5 Potential Benefits

Subjects will not personally benefit from participating in this clinical study, but there may be scientific benefit or benefit to the population at large. Although each subject will receive a YFV-17D vaccination booster, there is no guarantee that the booster will have a protective effect against YFV following administration. Subjects will be counseled to follow up with their primary care physician or a travel clinic for clinical travel recommendations prior to travel to a YFV endemic region.

5.9 Summary of Clinical Safety Data

1) YFV-17D is an FDA-approved vaccine introduced in 1936 and has a long history of safety in humans. Each year, it is routinely administered by the SC route to more than 200,000 civilians and more than 500,000 military personnel in the US and has been administered to more than 500 million people worldwide.
2) The most common AEs are mild flu-like symptoms.
3) Rare but severe and/or life-threatening allergic reactions (including anaphylaxis) are estimated to occur at a rate of ≤ 17 cases per million vaccinations in primary vaccination and 10-fold less often in booster vaccination.
4) Rare but severe and potentially life-threatening neurological disease (YFV-AND) requiring hospitalization is estimated to occur at a rate of 0.8 cases per 100,000 doses (all age groups) but is typically not lethal. There is a reduced risk of approximately 0.15 cases per 100,000 vaccinations in subjects aged 19 to 49. Booster doses account for ~3% of all cases.
5) Rare but severe and life-threatening viscerotropic disease (YEL-AVD) requiring hospitalization is estimated to occur in 0.4 cases per 100,000 vaccinations (all age groups) with an approximate 50% mortality rate, resulting in up to 2 fatalities per million vaccinations. There is a reduced incidence of approximately 0.07 cases per 100,000 vaccinations (9) to 0.24 cases per 100,000 vaccinations in subjects aged 19 to 49. Booster doses account for ~3% of all cases.

6 STUDY OBJECTIVES AND PURPOSE

The objective of this study is to use live attenuated yellow fever vaccine 17D as a live virus challenge to assess to mechanisms of immune protection in previously vaccinated adults aged 20-49 years old.

6.1 Primary Objective

To determine the pre-boost neutralizing antibody titer that correlates with sterile immunity, the most stringent measure of vaccine efficacy.

6.2 Secondary Objectives

To determine whether there is a measurable pre-vaccination antibody titer range in which vaccinees experience a memory antibody response as demonstrated by a four-fold rise in pre and post-vaccination antibody titers.
To determine whether there is an antibody titer below which subjects will develop a detectable vaccine viremia.

6.3 Exploratory Objectives

To determine which innate and adaptive immune responses are stimulated when a pre-immune subject is challenged with attenuated live yellow fever virus. To determine whether the stimulated immune responses correlate with antibody titer boosting or with detectable viremia.

7 STUDY DESIGN

7.1 Description of Study Design

This is a prospective single arm study of the immunogenicity of the yellow fever vaccine 17D (YFVax®, Sanofi Pasteur, Swiftwater, PA) in adults who have documented history of prior yellow fever vaccination 8 or more years prior. Study subjects will receive a single YFV-17D vaccination and be followed for 30 days after vaccination with blood samples collected for pre-screening and on vaccination days 0, 2, 4, 6, 8, 10, 12, 14 and 28 post vaccination. The study is designed to enroll 34 vaccinees at a single site, OHSU. Study duration is expected to be no longer than 60 days. Subject recruitment will be completed in 2 years.

7.2 Study Endpoints

7.2.1 Primary Endpoint

The primary endpoint will be fold change YFV neutralizing antibody titers levels measured on Day 0 and Day 28 post-vaccination.

7.2.2 Secondary Endpoints

- Vaccine viremia as measured by qRT-PCR, focus forming assay a virus isolation on days 0, 2, 4, 6, 8, 10, 12, and 14.

7.2.3 Exploratory Endpoints

- Frequency and magnitude $CD4^+$ and $CD8^+$ cell populations on day 0, 2, 8, 14, and 28 post vaccination.

- Multiplexed cytokine levels for 48 different cytokines measured on days 1, 2, 8, 14, and 28 days post vaccination.
- Titer of vaccination virus preparation as measured by qRT-PCR, focus forming assay and virus isolation.
- YFV specific MBC frequency for subject PBMCs collected on days 1, 2, 8, 14, and 28 days post vaccination.

7.2.4 Safety endpoints

- Frequency and percentage of subjects with solicited injection site reactions for 7 days post vaccination and solicited systemic events for 14 days post vaccination.
- Frequency and percentage of subjects with any unsolicited Adverse Events (AEs) up to 28 days post vaccination.
- Frequency and percentage of subjects with Medically Attended Adverse Events (MAAEs) throughout the trial.

7.3 Criteria for Premature Termination of the Study: Stopping Rules

Study enrollment and vaccination will be suspended pending review of all pertinent data after the occurrence of:

- 1 Grade 4 or 2 Grade 3 AEs possibly related to the vaccination
- 1 SAE possibly related to the vaccination (e.g. viscerotropic or neurological disease)

Adverse events will be graded according to the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials guidelines as outlined on the FDA website: http://fda.gov/cber/gdlns/toxvac.htm.
If the study is stopped due to meeting the above criteria, it may not be resumed until all pertinent data is reviewed by the Medical Monitor.

8 SELECTION AND WITHDRAWAL OF SUBJECTS

All entry criteria, including test results, need to be confirmed prior to first vaccination.

8.1 Inclusion Criteria

Subject eligibility is determined according to the following criteria:

1. Aged ≥20 to <50 years.
2. Male or female.
3. In good health at the time of screening as determined by medical history, physical examination, and clinical judgement of the investigator.
4. Documented history of Yellow fever vaccination 8 or more years prior. Documentation must be on a primary (not copied) vaccination card or a fully completed electronic medical record entry including date of administration and lot number administered.
5. Subjects who can comply with all trial procedures and are available for the duration of follow-up.

8.2 Exclusion Criteria

Any subject who meets any of the following criteria will not qualify for study enrollment:

1. A clinically active infection or self-reported body temperature ≥38°C (100.4°F) within 3 days of scheduled date of vaccination (consider whether the finding is an exclusion criterion or criterion for delay of vaccination see Section 8.3).
2. A known hypersensitivity or allergy to any of the trial vaccine components including eggs.
3. Behavioral/cognitive impairment that, in the investigator's opinion, may interfere with the subject's ability to participate safely in the trial.
4. Any history of neurologic disorder, seizure disorder or neuro-inflammatory disease.
5. Any illness, or history of any illness that, in the investigator's opinion, could interfere with the trial or pose an additional risk to the subject during the trial period.
6. Known or suspected impairment/alteration of immune function, including:
    a) Chronic use of oral steroids within 60 days prior to enrollment. Inhaled steroids are allowed.
    b) Receipt of parenteral steroids within 60 days prior to screening visit.
    c) Receipt of immunoglobulins and/or any blood products within the 3 months prior to enrollment or planned receipt during the trial.
    d) Receipt of immunostimulants within 60 days prior to screening visit
    e) Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months of enrollment.
    f) Known Human Immunodeficiency Virus (HIV) infection or HIV-related disease.
    g) Hepatitis C virus infection.
    h) Genetic immunodeficiency.
7. History of splenic or thymic dysfunction.
8. Any serious chronic or progressive disease as assessed by the investigator (eg, neoplasm, hematologic malignancies, insulin dependent diabetes; cardiac, renal, or hepatic disease).
9. Body Mass Index (BMI) greater than or equal to 35 kg/m^2.
10. Concurrent participation in any clinical trial with another investigational product 30 days prior to or during the conduct of this trial.
11. Vaccination within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to enrollment or plans to receive any vaccine within 28 days of trial vaccine administration (consider whether applicable as an exclusion criterion or criterion for delay of trial vaccine administration).
12. Use of antipyretics and/or analgesic medications within 24 hours prior to vaccination. Trial entry should be delayed to allow for a full 24-hours to have passed since last use of antipyretics and/or analgesic medications (consider whether applicable as an exclusion criterion or criterion for delay of trial vaccine administration).
13. Subjects with history of substance or alcohol abuse within the past 2 years.
14. Subjects who are pregnant or breastfeeding.
15. Subjects of childbearing potential who are sexually active with men and have not used "acceptable contraceptive methods" for at least 2 months prior to enrollment.
    a) Of "childbearing potential" is defined as beyond onset of menarche and not: menopausal for 2 or more years, post bilateral tubal ligation at 1 year prior, post bilateral oophorectomy for at least 1 year or post hysterectomy.
    b) "Acceptable birth control methods" include:
        a. Hormonal contraceptives (such as oral, injection, transdermal patch, implant, cervical ring).
        b. Barrier method (condom with spermicide or diaphragm with spermicide) every time during intercourse.
        c. Intrauterine device.
        d. Monogamous relationship with vasectomized partner (partner must have been vasectomized for at least 6 months prior to the subject's enrollment.

16. Subjects of childbearing potential who are sexually active with men and refuse acceptable contraceptive method up to 28 days after the vaccination.
17. Any positive or indeterminate pregnancy test.
18. Planned vaccination (during the trial conduct) against any other vaccine preventable disease.
19. Planned travel (during the trial) to any YFV endemic area.
20. Screening serology consistent with prior history of dengue, zika, West Nile or Japanese encephalitis virus infection.
21.

It may occur that a prospective subject meets all entry criteria except one that relates to short term clinical condition (e.g., fever, recent use of excluded medications). Under these circumstances, eligibility for delayed trial enrollment may be considered after inclusion/exclusion criteria have been rechecked, and if the subject is confirmed to be eligible.

8.3 Criteria for delay

After enrollment, subjects may temporarily encounter circumstances that warrant delay in vaccination. If a subject meets a criterion for delay of vaccination, the subject may still be vaccinated after the window for delay has passed as long as the subject otherwise remains eligible for trial participation.

The following clinical circumstances warrant a delay for administration of the vaccination:

- Subjects with a clinically active infection (as assessed by the investigator) or body temperature ≥38°C (100.4°F) within 3 days of the vaccination date.
- Subjects who have received, or have scheduled, any other vaccines within 30 days for both inactivated and live vaccines prior to trial vaccination.
- Subjects who have used antipyretics and/or analgesic medications <=24 hours prior to vaccination. Trial vaccination should be delayed to allow for 24-hours to pass between having used antipyretics and/or analgesic medications and trial vaccine administration.

8.4 Early Termination of subject trial participation
Under certain circumstances, a subject's trial participation may be terminated early. This means that no further trial procedures (including data collection) will be performed on that subject beyond the date of early termination of trial participation. The primary reason for early termination of the subject's trial participation should be documented using the following categories. Although subject is not obligated to provide a reason for withdrawing, attempts should be made to determine the reason for the withdrawal and, where possible, the underlying reason should be documented.

1. Adverse Event: The subject has experienced an AE (related/unrelated to the trial vaccination or trial-related procedures) that imposes an unacceptable risk to subject health and/or the subject is unwilling to continue participation because of the AE. In these cases, the primary reason for early termination of trial participation in this case will be 'withdrawal due to AE'. Any ongoing AEs leading to early termination of trial participation should be followed up by the investigator until resolution or stabilization and safety follow-up will be offered.
2. Lost to follow-up: The subject did not return to the trial site and at least 3 attempts to contact the subject failed.

3. Withdrawal of consent: The primary reason for early termination will be "withdrawal of consent" if the subject withdraws for a non-medical reason (i.e., reason other than AE). The reason for withdrawal, if provided, should be documented.
4. Premature trial termination by a regulatory agency, the IEC/IRB, or any other authority.
5. Subject's death during trial participation.

9 TREATMENT OF SUBJECTS

9.1 Study Vaccine and Dosing Regimen

YF-VAX® (YFV-17D) is manufactured and distributed by Sanofi Pasteur Inc. (Swiftwater, Pennsylvania). YF-VAX® is formulated to contain not less than 5.5 x 104 PFU/0.5 mL dose in single-dose or multiple-dose (5-dose) vials reconstituted with 0.6 mL or 3 mL of diluent, respectively (see YF-VAX® package insert for details).

Storage

YF-VAX® will be shipped frozen in a container containing solid carbon dioxide (CO2; dry ice) and will not be used if the shipping case contains no dry ice upon arrival. Upon receipt, lyophilized vaccine must be maintained continuously at 2˚C to 8˚C. The vaccine will not be used after the expiration date. The vaccine will not be re-frozen. YF-VAX® does not contain a preservative; all vaccine must be used within 1 hour after reconstitution.

Vaccine Preparation

The research pharmacist will reconstitute the vaccine per manufacturer's instructions: YF-VAX® will be diluted to deliver not less than 5.5 x 104 PFU/0.5 mL dose in single-dose using the diluent supplied (sodium chloride for injection USP). The reconstituted vaccine should sit at room temperature for 1 to 2 minutes and then be carefully swirled until a uniform suspension is achieved. The reconstituted vaccine will appear slightly pink-brown in color. If the product contains extraneous particulate matter or is discolored, do not administer the vaccine. The vaccine will be swirled well and administered within 1 hour after reconstitution.

Administration and Dosage

Subcutaneous vaccination will deliver the standard 0.5 mL dose in the subject's preferred deltoid. Tattoos and other skin findings that may obscure injection site reactions will be avoided.

9.1.1 Interruption of Study Drug

If unexpected AEs are noted, administration of YF-VAX® may be interrupted or discontinued. Dose modification is an unlikely course of action because the SC dose is the FDA-approved standard concentration.

9.1.2 Vaccine Toxicity Management

The clinical investigator will monitor all subjects for any vaccine-associated toxicity.
The following plan has been developed to monitor subjects with severe or life-threatening vaccine-associated toxicities:

- Study subjects will maintain a paper diary for local (injection site) for 7 days and systemic symptoms for 14 days post vaccination. Subjects will receive a thermometer and will record document temperatures at least once daily and more frequently if they experience a subjective fever.

- The study visit schedule ensures that subjects are seen by the investigator every other day +/- from post vaccination day 0 through 14.
- At each visit, diary cards will be reviewed and a focused physical exam will be performed by the investigator as indicated by the schedule of events.
- Liver function tests will be performed at post-vaccination days 0, 6, 14 and 28 to detect any abnormalities.
- The investigator will be prepared to refer any subject requiring specialized treatment to a medical specialist (e.g., neurologist, gastroenterologist).

9.2 Documentation of Study Product

The vaccine, YF-VAX®, will be administered at Oregon Health & Science University (OHSU), under the supervision of the PI. Careful logs will be kept to monitor receipt, dispensation, and destruction of drug through the pharmacy at each institution. The investigator is required to maintain adequate records of the disposition of the vaccine and placebo, including dates, quantity, and use by subjects as outlined in 21 Code of Federal Regulation (CFR) 312.62. If the investigation is terminated, suspended, discontinued, or completed, the investigator must destroy the unused supplies of the vaccine as required under 21 CFR 312.59.

9.5 Subject Compliance

Compliance will be monitored by study personnel who will administer the vaccine. Subjects who miss a scheduled visit will be contacted by phone, text or email, and the visit will be re-scheduled.

10 STUDY PROCEDURES

10.1 Trial Procedures

The following sections describe the trial procedures and data to be collected. All procedures must be performed by qualified and trained staff.

10.1.1 Informed consent

- Informed consent must be obtained prior to the subject entering into the trial, and before any protocol-directed procedures are performed. Non-English speaking participants will not be targeted but should the need arise to consent someone that is non-English speaking, a short form and interpreter services will be used. A modification will be submitted for approval of the needed short form. A unique subject number will be assigned to each subject after informed consent is obtained. If all eligibility criteria are fulfilled, this subject number will be used throughout the trial. Subject numbers assigned to subjects who fail screening should not be reused.

10.1.2 Demographics, Medical History and Prior Medications

- Demographic information will include age, sex, race, and ethnicity as described by the subject.
- Medical history will also be collected, including but not limited to any medical history that may be relevant to subject eligibility for trial participation such as prior vaccinations, current medications, and previous and ongoing medical conditions. Relevant medical history may also include anything that contributes to the understanding of an AE that occurs during trial participation, if it represents an exacerbation of an underlying disease/preexisting problem.
- Medical history will include any significant conditions or diseases that have disappeared or resolved at or prior to signing of the informed consent form.

- All medications, vaccines and blood products taken by the subjects are to be collected and documented in the subject's source document:
  a) Medications: from 1 month (minimum 28 days) prior to vaccination and up to 1 month thereafter.
  b) Steroids and immunostimulants within 60 days prior to enrollment.
  c) Immunoglobulins and blood products within 3 months prior to enrollment.
  d) Immunosuppressive therapy within 6 months prior to enrollment.
- The use of antipyretics and/or analgesic medications <= 24 hours prior to noted and the reason for their use documented in the subject's source documents. Vaccination should be delayed if subjects have used antipyretics and/or analgesic medications within 24 hours of scheduled vaccine administration.
- Prohibited therapies:
  a) Parenteral immunoglobulin, blood products, and/or blood-derived products within the 3 months prior to enrollment.
  b) Immunosuppressive therapy within 6 months or systemic corticosteroid treatment within 60 days prior to enrollment,
  c) or immunostimulants within 60 days prior to enrollment.
  d) Any other vaccines within 30 days prior to vaccination and 30 days after vaccination.
  - Serological testing to ensure no prior Flavivirus infection.

  These data must be written in the subject's source documents.

10.1.3 Documentation of Trial Entrance/Randomization

- Subjects who have a signed informed consent form, meet all of the inclusion criteria and none of the exclusion criteria are eligible for entrance into the active phase.
- If the subject is found to be ineligible for enrollment, the investigator should record the primary reason for failure on the subject's screening and enrolment log.
- Randomization is not applicable for this open-label trial.

10.1.4 Physical Examination

- A complete physical examination must be performed by a qualified health professional in accordance with local regulations prior to vaccination. A complete physical examination includes but is not limited to: auscultation of heart and lungs, palpation of the abdomen, inspection of extremities (including skin over intended vaccination site), a check of general appearance and the measurement of weight and height; BMI will be calculated. Additional physical examinations may be performed if indicated by subject's medical history. The findings should be documented in the subject's source document.
- Targeted symptom-directed physical examination including but not limited to measurement of vital signs may be performed on visit days 2, 4, 6, 8, 10, 12, and 14. Clinically significant changes, as determined by the investigator, from the baseline examination should be recorded in the subject's source documents.

10.1.5 Vital Signs

- During the physical examination, a subject should have their vital signs measured, including (but not limited to) the measurement of systolic blood pressure/diastolic blood pressure, heart rate, and body temperature at all trial visits.

10.1.6 Laboratory tests

- Baseline complete blood count with differential and complete metabolic panel will be drawn on enrollment visit.

10.1.7 Immunogenicity Assessments

- Vaccine viremia will be measured using blood samples collected on post vaccination day 0, 2, 4, 6, 8, 10, 12, and 14.
- Yellow fever neutralizing antibodies will be measured using blood samples collected from all subjects prior to first vaccination on day 0 and day 28.

- T and B cell-mediated immune response will be measured using blood samples collected from all subjects collected post vaccination day 00, 2, 8, 14, and 28.
- The innate immune response will be measured using blood samples collected post-vaccination days 0, 2, 8, 14 and 28.
- All blood samples will be processed, labeled and stored according to the Laboratory Manual or other appropriate guideline provided to the trial site.

10.1.8 Processing, Labeling and Storage of Biological Samples

- PBMC will be collected, processed, labeled and securely stored according to laboratory Standard Operating Procedures (SOP).
- All biological samples will be processed, labeled and stored according to SOP or other appropriate guideline provided to the site.

10.1.9 Safety Assessments

- Safety assessments will include collection and recording of solicited local (injection site) reactions and solicited systemic events, unsolicited AEs (serious and non-serious), and

10.1.10 Clinical Safety Laboratory Variables

- Liver function tests will be performed on post vaccination days 0, 6, 14, and 28

10.1.11 Contraception and Pregnancy Avoidance Procedure

- For female subjects of child bearing potential, point of care urine pregnancy testing will be performed prior to vaccination (Day 0). Results must be confirmed and documented as negative prior to vaccination. Subjects will be provided with information on acceptable methods of contraception as part of the subject informed consent process and be asked to sign a consent form stating that they understand the requirements for avoidance of pregnancy and donation of ova.

10.2 Monitoring Subject Compliance

The investigator must record all injections of trial vaccine given to the subject in the subject's source document.

10.3 Schedule of Observations and Procedures

10.3.2 Pre-Vaccination Procedures

1. Before performing any trial procedure, the signed informed consent form needs to be obtained.
2. Check inclusion and exclusion criteria.
3. Review demographic data, medical history, and prior medication/vaccination.
4. Review of systems: a structured interview that queries any complaints the subject has experienced across each organ system.
5. Perform a complete physical examination.
6. Check vital signs.
7. Perform pregnancy testing (serum or urine) for females of childbearing potential.
8. Provide avoidance of pregnancy guidance for females of childbearing potential who are sexually active.
9. Collect pre-vaccination blood samples

10.3.3 Vaccination Procedures

1. Check criteria for delay of trial vaccine administration.
2. Check contraindications for trial vaccine administration.
3. Administer the trial vaccine.

10.3.4 Post Vaccination Procedures

1. Review with the subject how to measure solicited local (injection site) reactions and body temperature, how to complete the diary card and how often to complete the diary card. Training of the subject on how to measure an injection site reaction and how to take their temperature, as well as how to record the information in the diary card, should be performed while the subject is under observation after vaccination.

2. The subject must understand that timely, daily completion of the diary card is a critical component of trial participation. Subjects should be instructed to write clearly and to complete the diary card in pen. Any corrections to the diary card by the subject should include a single strikethrough line with a brief explanation for any change and be initialed and dated. Diary cards will be the only source document for remote collection of solicited local (injection site) reactions, solicited systemic events (including body temperature measurements), and unsolicited (non-serious) AEs.
3. The diary card should be reviewed with the subject.
4. No corrections or additions to the diary card will be allowed after it is reviewed by the investigator.
5. Any data that is identified as implausible or incorrect, and confirmed by the subject to be a transcription error, should be corrected by the subject on the diary card with a single strikethrough line and should be initialed and dated by the subject.
6. Any newly described solicited and unsolicited (non-serious) safety information should be added to the diary card by the subject, initialed, and dated.
7. Starting on the day of vaccination, the subject will check for specific types of events at the injection site (solicited local [injection site] reactions), any generalized symptoms (solicited systemic events) including body temperature, unsolicited (non-serious) AEs, any other change in the subject's health status, and any medications taken. Solicited local (injection site]) reactions, solicited systemic events, body temperature, and unsolicited (non-serious) AEs will be recorded in the diary. Assessments should preferably take place at the end of the day.
8. Body temperature measurement is to be performed using a thermometer provided by the study team. Temperatures should be taken at approximately the same time of day, preferably at the end of the day. If the subject feels unusually hot or cold during the day, the subject should check his/her temperature. If the subject has fever, the highest body temperature observed that day should be recorded on the diary card.
9. The measurements of solicited local (injection site) reactions are to be performed using the ruler provided by the study team.
10. The collection on the diary card of solicited local (injection site) reactions, solicited systemic events (including body temperature measurement), and unsolicited (non-serious) AEs will continue for a total of 7 days, 14 days, and 28 days following vaccination. Any solicited local (injection site) or systemic AE that resolves before 8 or 15 days, respectively, following each trial vaccination, but recurs at a later time (ie, if discontinues), should be recorded as an unsolicited AE on the "Adverse Event".
11. After each trial vaccination, the subject will be observed for at least 30 minutes including observation for solicited local (injection site) reactions, solicited systemic events (including body temperature measurement), and unsolicited AEs. The investigator or delegate will take the opportunity to remind the subject how to measure solicited local (injection site) reactions and body temperature as part of this observation period. All safety data will be collected in the subject's source documents.
12. The site should schedule the next trial visit with the subject.
13. The subject will receive a written reminder of the next trial visit.
14. The subject will be reminded to complete the diary card daily, to contact the site if there are any questions, and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit or is otherwise perceived as serious. All contact details will be provided to the subject.

10.3.5 Site Visits after Vaccination: Days 2, 4, 6, 8, 10, 12, 14, 28.

1. Review the diary card with the subject days 2, 4, 6, 8, 10, 12, 14.
2. Collect and record persistent/prolonged solicited local (injection site) reactions.

3. Collect and record persistent/prolonged solicited systemic events.
4. Collect concomitant medications/vaccinations.
5. Perform a complete physical examination on day 28.
6. Check vital signs.
7. Provide guidance with respect to the avoidance of pregnancy for females of childbearing potential who are sexually active.
8. Collect and report SAEs.
9. Collect blood samples. Blood should be taken from the subject using an aseptic venipuncture technique.
10. The investigator or delegate should schedule the next trial visit with the subject, as applicable.
11. The subject will receive a written reminder of the next trial visit, as applicable.
12. The subject will be reminded to complete the diary card daily and to contact the site if there are any questions, and to contact the site immediately if the subject has a medical condition that leads to a hospitalization or an emergency room visit or is otherwise perceived as serious. All contact details will be provided to the subject.

10.3.6 Final (End of Trial) Visit

The final (end of trial) visit will be performed on Day 28. If a subject terminates earlier, the final (end of trial) visit procedures should be performed at their last trial visit, if possible.

10.3.7 Post-Trial Care

No post-trial care will be provided.

10.4 Sample Collection

10.4.1 Blood Samples

Primary and secondary endpoint assessments and clinical safety labs will be performed using blood samples collected at each study visit. All blood samples will be processed, labeled and stored according to the appropriate guideline provided to the site. Samples will be preserved and retained at a central laboratory indefinitely or as required by applicable law. The Investigator has put into place a system to protect the subject's personal information to ensure optimal confidentiality and defined standard processes for sample and data collection, storage, analysis, and destruction.

10.4.2 PBMC Cryopreservation

The blood samples obtained on post vaccination days 0, 2, 8, 14 and 28 will be a source of PBMCs and serum for studies involving innate and cellular immune responses to viral infection. PBMCs from heparinized whole blood will be collected and cell numbers will be determined. Multiple aliquots will be appropriately labeled with a unique identification code, cryopreserved, and stored in liquid nitrogen.

11 SAFETY ASSESSMENTS

11.1 Safety Assessments Overview

Adverse events that are classified as serious (see Section 11.2.3) according to the definition of 21 CFR 312.32 must be reported promptly and appropriately to institutional review boards (IRBs) and health authorities. This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording, and reporting them.

Information in this section complies with ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Good Clinical Practice (GCP) and applies the standards set forth in the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials guidelines (September 2007)(33). This document provides a common vocabulary for describing grades of severity to analyze and interpret data, and for articulating the clinical significance of all AEs. The Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials guidelines (September 2007) have been reviewed by the PI and have been deemed appropriate for the subject population to be studied in this protocol.

Subjects will be asked about signs and symptoms at each clinical encounter. Clinical laboratory data will be analyzed to address safety issues. Physical examinations will be performed and vital signs collected (including: heart rate, respiration rate, body temperature, blood pressure).

11.2 Definitions

11.2.1 Adverse Events

An AE is any occurrence or worsening of an undesirable or unintended sign (including an abnormal laboratory finding), symptom, or disease that is temporally associated with the use of a study product, whether considered related to the study product or not.

Expected AEs: Adverse events associated with YF-VAX® may include redness, pain, and swelling at the injection site, mild headaches, myalgia, low-grade fevers, or other minor symptoms for 5 to 10 days post-vaccination. Local reactions — including edema, hypersensitivity, pain, or mass at the injection site — have also been reported following YF vaccine administration. Immediate hypersensitivity reactions — characterized by rash, urticaria, and/or asthma — are uncommon and occur principally among persons with a history of egg allergy. Approximately 72% of subjects experienced non-serious AEs judged to be related to vaccination. Most of these were injection site reactions of mild to moderate severity.

Solicited local (injection site) reactions and systemic AEs

- Local reactions
    - Pain
    - Erythema
    - Swelling
- Systemic events
    - Fever
    - Headache
    - Asthenia
    - Malaise
    - Myalgia
    - Rash

Severity scale for solicited Safety Parameters

- Pain at injection site
    - 0 None
    - 1 Mild – no interference with activities of daily living (ADLs)
    - 2 Moderate – interference with ADLs without treatment
    - 3 Severe – prevents ADLs without treatment

- Erythema at injection site
  - 0 <25 mm
  - 1 Mild: ≥25-≤50 mm
  - 2 Moderate: >50-≤100 mm
  - 3 Severe: >100mm
- Swelling at injection site
  - 0 <25 mm
  - 1 Mild: ≥25-≤50 mm
  - 2 Moderate: >50-≤100 mm
  - 3 Severe: >100mm
- Headache
  - 0 None
  - 1 Mild – no interference with activities of daily living (ADLs)
  - 2 Moderate – interference with ADLs without treatment
  - 3 Severe – prevents ADLs without treatment
- Asthenia
  - 0 None
  - 1 Mild – no interference with activities of daily living (ADLs)
  - 2 Moderate – interference with ADLs without treatment
  - 3 Severe – prevents ADLs without treatment
- Malaise
  - 0 None
  - 1 Mild – no interference with activities of daily living (ADLs)
  - 2 Moderate – interference with ADLs without treatment
  - 3 Severe – prevents ADLs without treatment
- Myalgia
  - 0 None
  - 1 Mild – no interference with activities of daily living (ADLs)
  - 2 Moderate – interference with ADLs without treatment
  - 3 Severe – prevents ADLs without treatment

11.2.2 Medically Attended Adverse Events

MAEEs are defined as AEs that result in an unscheduled medical visit by a healthcare professional, including visits to an emergency department, but not fulfilling seriousness criteria.

11.2.3 Serious Adverse Events

An SAE or reaction is defined as “any AE occurring at any dose that suggests a significant hazard, contraindication, side effect, or precaution.” This includes, but is not limited to, any of the following events:

1) Death: A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up period after the completion of therapy must be reported to the independent safety monitor (ISM) whether it is considered treatment related or not.

2) A life-threatening event: A life-threatening event is any adverse therapy experience that, in the view of the investigator, places the subject at immediate risk of death from the reaction as it occurred.

3) Inpatient hospitalization or prolongation of existing hospitalization

4) Persistent or significant disability/incapacity

5) Congenital anomaly or birth defect

An important medical event that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Serious AE reporting will begin with events that arise after the informed consent is signed until 30 days after the last study visit.

11.2.4 Unexpected Adverse Events

An AE is considered “unexpected”, when the nature (specificity) or severity of the AE is not consistent with applicable product information, such as safety information provided in the package insert.

11.3 Collection and Recording of Adverse Events

11.3.1 Collection Period

Adverse events will be collected from the time the subject signs the study consent until 30 days after he/she completes study participation, or until 30 days after he/she prematurely withdraws from the study.

Once recorded, an AE will be followed until it resolves with or without sequelae, becomes medically stable, or until 30 days after a participant completes or terminates from the study, whichever comes first. Where indicated, particularly in the event of neurological, gastrointestinal, or cardiac problems, the subject will be referred to appropriate medical specialists.

11.3.2 Collection of Adverse Events

Adverse events (including SAEs) may be discovered through any of these methods:
- Observing the subject
- Questioning the subject in an objective manner
- Receiving an unsolicited complaint from the subject
- Reviewing subject diary information

In addition, an abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an AE. If this is the case, then the evaluation that produced the value or result should be repeated until that value or result returns to baseline or can be explained and the subject’s safety is not at risk. If an abnormal value or result is determined by the investigator to be clinically significant, it must be recorded as an AE.

11.3.3 Recording Adverse Events

Throughout the study, the investigator will record and grade AEs using the appropriate source document and will enter it into the system regardless of event severity or relation to study

product or study procedure. All requested information on the source document should be provided and entered, if available.

For a fatal outcome, cause of death and a comment on its possible relationship to the study treatment should be provided. For each AE or SAE, one of the following outcomes will be determined:

- Ongoing stable
- Ongoing unstable
- Resolved without sequelae
- Resolved with sequelae (this could also be stability)
- Death

Actions taken in response to an AE and follow-up results (including lab results) will also be recorded in the subject's medical record in accordance with local procedure. Any treatment administered for the AE must be recorded in the subject's EDC source document and entered into the EDC system. When a subject is discontinued from the study due to an AE, relevant clinical assessments and laboratory tests will be repeated as necessary until final resolution, stabilization, or until 30 days after discontinuation occurs, whichever comes first.

11.3.4 Recording Serious Adverse Events

Serious AEs will be recorded in REDCap, the EDC, on the Serious Adverse Event Report Form, which will then be sent to the medical monitor about the SAE (Section 11.5.2). In addition, SAEs will be recorded in the EDC source document and entered into the EDC system. All requested information on the AE EDC form should be provided, if available.

11.4 Grading and Attribution of Adverse Events

11.4.1 Grading Criteria

The study site will grade the severity of AEs experienced by the study subjects according to the criteria set forth in the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials guidelines:

Adverse events will be graded on a scale from 1 to 4 according to the following standards in the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials guidelines:
Grade 1 = mild AE.
Grade 2 = moderate AE.
Grade 3 = severe and undesirable AE.
Grade 4 = life-threatening AE, disabling AE, or death.

11.4.2 Attribution Definitions

The relation, or attribution, of an AE to a vaccine will be determined by the site investigator. The site investigator will also record the determination of attribution on the appropriate EDC source document, entered into the EDC system, and/or SAE reporting form. The relation of an AE to the vaccination or study procedures will be determined using the descriptors and definitions provided in Table 11.1.

Table 11.1 Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials Guidelines Attribution of Adverse Events.

Code	Descriptor	Definition
Unrelated Category		
1	Unrelated	The AE is clearly not related to the vaccination.
Related Categories		
2	Unlikely	The AE is doubtfully related to the vaccination.
3	Possible	The AE may be related to the vaccination.
4	Probable	The AE is likely related to the vaccination.
5	Definite	The AE is clearly related to the vaccination.

For additional information and a printable version of the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials guidelines, consult the FDA website: http://fda.gov/cber/gdlns/toxvac.htm.

11.5 Reporting of Adverse Events

11.5.1 Adverse Events Requiring Expedited Reporting

The following AEs, as graded with the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials guidelines (Section 11.4.1) will be reported in an expedited fashion by investigators to the institutional review board of receipt (Section 11.5.2), regardless of expectedness or relationship to the vaccination:

- All SAEs per 21 CFR 312.32 definitions (Section 11.2.3)
- All Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials guidelines Grade 3 and 4 (33)
- Any event that the investigator considers serious but is not easily categorized

11.5.2 Reporting Timeline

The following process for reporting an SAE ensures compliance with ICH guidelines. When an investigator identifies an SAE (as defined in Section 11.2.3) or other AE requiring expedited reporting (see Section 11.5.1), the ISM must be notified immediately upon discovery of the event, and must complete and send the Serious Adverse Event Form within 1 business day to the Independent Safety Monitor within 2 business days after receipt of the SAE report from the site.

All non-serious AEs (defined in Section 11.2.1) will be recorded on the appropriate EDC source document and entered into the AE form included in the EDC system. Non-serious AEs will be reported based on the guidelines of the institutional review board.

The site investigator will follow the progress of a subject who experiences a SAE until the SAE is resolved, considered stable, or until 30 days after the subject completes or terminates from the study, whichever comes first. If the unexpected SAE has not resolved by the report deadline, the investigator will send follow-up reports.

11.5.3 Reporting of Adverse Events

All AEs (serious or otherwise) All SAEs will be reported per YFVax package insert, "To report SUSPECTED ADVERSE REACTIONS, contact the Pharmacovigilance Department, Sanofi

Pasteur Inc., Discovery Drive, Swiftwater, PA 18370 at 1-800-822-2463 (1-800VACCINE) or VAERS at 1-800-822-7967 or https://vaers.hhs.gov.

11.5.4 Reporting Pregnancy as a Serious Adverse Event

This study requires that any pregnancy be reported as an SAE manufacturer for tracking purposes only. All pregnancies identified during the study must be followed to conclusion and the outcome must be reported. The investigator should be informed immediately of any pregnancy in a study subject. The investigator should report all pregnancies to the ISM within 1 business day (see Section 11.5.2). The investigator should counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject should continue until the conclusion of the pregnancy, and a follow-up SAE reporting form detailing the outcome of the pregnancy should be submitted.

Information requested about the delivery will include:

- Subject's enrollment identification
- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender
- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any congenital abnormalities

11.6 Review of Safety Information

11.6.1 Routine ISM Review

The ISM will generate monthly reports that compile all newly submitted and accumulated AEs and SAEs. Subsequent reviews of the periodic reports will be performed by the FDA.

11.6.2 ISM Product Safety Associate Review

The ISM will review all SAEs immediately upon notification by the Investigator. These reports will consist of all AEs and SAEs.

11.6.3 Independent safety monitor. An independent safety monitor will be identified as mandated by the OHSU IRB in data safety and monitoring plan (DSMP).

11.6.4 Institutional Review Board Reporting of Adverse Events

The investigator must report AEs to OHSU IRB as locally mandated.

11.7 Protocol Deviations

The study coordinator will report all protocol deviations within 1 business day by completing the EDC Protocol Deviation Form. This form will ask for a description of the event and what corrective action is planned. All protocol deviations that meet reporting criteria will be reported to the OHSU IRB within 5 business days.

Protocol deviations occur under the following circumstances: when non-adherence to the protocol results in a significant added risk to the study subject; when the study subject or investigator has failed to adhere to significant protocol requirements; or when there is non-adherence to GCP guidelines. NIAID will not allow waivers or planned deviations of enrollment criteria; enrollment of subjects who do not meet the inclusion criteria constitutes a deviation from the protocol.

11.8 Clinical Monitoring

Study staff unrelated to any other aspect of the trial will monitor the study to verify that the rights and well-being of the subjects enrolled under this protocol are protected and that the data being collected by the site are accurate, complete, and verifiable from source documents. The study PI will verify that the site complies with the currently approved protocol and any protocol amendments, and with GCP, and any other regulatory requirements. The site will be monitored in accordance with the monitoring plan developed for this clinical study.

12 DATA HANDLING AND RECORD KEEPING

12.1 EDC and Source Documentation

The site study coordinator will complete an EDC source document for each subject. Completion instructions for the EDC source document are embedded in the document and an online help function is provided to assist with EDC entry. Information in the EDC system must be currently maintained and up-to-date.

Subjects must not be identified by name on any study document. Subjects will be identified by subject identification numbers assigned at enrollment.

12.2 Data Management

Data for this project will be collected and stored in OCTRI's installation of REDCap, a highly secure and robust web-based research data collection and management system. Using this system, clinical site personnel use an internet browser to key data into electronic data fields. Univariate data validation tests will be performed as the data are keyed, and most implausible data values will be resolved immediately. Authorized site personnel may log in to the system at any time, review and correct previously entered data, and key additional data.

Adverse events will be coded as detailed in the safety monitoring plan.

Upon completion of processing, the operational database will be subjected to database closure procedures and subsequently locked. The PI will sign-off on eCRFs after review.

After the operational database is locked, the programming team will create and validate an analysis database. The analysis database will be the basis for statistical analyses of the data.

> *Physical security*: The KPNW CHR and OHSU OCTRI laboratories (location of specimen storage freezers) and the offices of research personnel have limited access. Additionally, the PI’s and research staff’s office doors are kept locked when they are not there. The OHSU OCTRI and Messer Laboratories are in locked rooms with access limited to research personnel. OCTRI's REDCap software is housed on servers located in ITG's Advanced Computing Center providing locked physical security.

Electronic security: Associated data will be held securely in compliance with OHSU and KPNW Information and Security Guide. Specifically, data will be held on OHSU networked drives, behind the OHSU firewall. Files will be password-protected. The REDCap servers are housed behind both the OHSU firewall and a second ACC firewall. All web-based data transmissions are encrypted with industry-standard SSL methods.

Controlled User Access: REDCap employs a robust multi-level security system that enables researchers to easily implement "minimum necessary" data access for their research staff, including specification of data fields that are identifiers. This feature includes “single click” ability to provide completely deidentified (removing all identified data fields and shifting dates) for analysis or other purposes. User activities are logged to enable auditing of all data access. Access is integrated with OHSU's network such that users who are also OHSU employees are authenticated against their OHSU network credentials.

Data Integrity: REDCap is jointly managed in accordance with OHSU Information Security Directives by ACC staff and members of OCTRI's Biomedical Informatics Program, ensuring fidelity of database configuration and back-ups. User activities are logged to enable auditing of all data changes.

12.3 Data Monitoring

After study initiation, clinical research associates will monitor EDC system data and source documents, as well as, compliance with the protocol, and subject safety.

12.4 Record Retention

The study-related records will be maintained at the site using either OHSU’s electronic medical record, EPIC, or Florence eBinders, which is a 21 CFR Part 11 compliant database to be used for clinical trial research. These records will include the following:

1) Copies of the protocol and all amendments
2) Signed Investigator Page for the protocol and all amendments
3)
4) Approval letter(s) from, and all other correspondence to and from, the IRB
5) Curricula vitae (CVs)(current within 2 years at study initiation) and current medical licenses for the PI and any sub-investigators
6) Delegation log of all study personnel
7) The original signed informed consent form for each subject screened and signed consent forms
8) Copies of results of all laboratory tests and other original data from which CRF information was obtained, as well as, laboratory normal ranges and certifications, when indicated
9) Copies of all correspondence relating to this investigation
10) Copies of financial disclosure documents

13 STATISTICAL ANALYSIS

13.1 Analysis Populations

13.1.1 Analysis Population

All eligible, enrolled, and randomized subjects who have both a baseline antibody titer (obtained during the Screening Visit) and a Day 28 titer will be included in the Analysis population.

13.2 Statistical Methods

13.2.1 Demographics, Baseline Characteristics, and Disposition

Background and baseline demographic, physical examination, and subject disposition data will be tabulated and presented for all enrolled subjects. Disposition will be tabulated according to the number (%) of subjects who have: signed informed consent, provided baseline/screening information, provided baseline blood draw for YF titer, completed each of the follow-up study visits for blood draw, and completed Day 28 (Visit 8) study procedures. Continuous data (e.g., age, body weight, height) will be summarized descriptively by mean, standard deviation (SD), median, and range. Categorical data (e.g., gender, race) will be presented as enumerations and percentages.

13.2.2 Primary Analysis –

Fold change between pre and post-vaccination neutralizing antibody titer. Subject serum is serially diluted four-fold from starting dilutions of 1:5 and mixed with an equal volume of a fixed number of plaque or focus forming units (FFU) added to 96 wells plates seeded with Vero cells 24 hours prior, and overlaid with methylcellulose. After 3 days incubation overlay is removed, cells are fixed and immune-stained, visualized, and counted on a CTL Immunospot™ S6Macro automated plate reader. Experiments will be conducted in 3 biologic replicates to ensure stable titers estimates. Foci counts are quality controlled by a technician blinded to sample exposure history. Positive and negative control sera will be run with each set of unknown sera. Foci counts will be transformed into percent neutralization by serum dilution and NT_{90} values will be calculated by sigmoid dose-response curve fitting.

13.2.3 Secondary Analyses

Viral load. Quantitative reverse-transcription PCR will be used to quantify viral genomic copies/mL in the vaccine inoculum and acute (Days 2-14) post-vaccination sera as previously described[46,63] modified for 96-well high-throughput using the Qiacube™ sample preparation robot in the OHSU Gene Profiling Shared Resource (GPSR) for RNA extraction quantified using the QuantStudio ™ 12K Flex instrument supported by the GPSR. Virus quantification will also be done simultaneously on both vaccine inoculum and acute sera by direct plaque assay as previously described[55], modified for 96-well plates, and virus isolation in tissue culture. To ensure rigor and reproducibility, all technicians will be blinded to subject study ID, known copy number positive controls and virus controls will be run with each batch.

Area under the curve receiver operator characteristic (AUC ROC). We will conduct AUC ROC cutoff analyses to identify post-hoc the most sensitive and specific cutoff NT_{90} serum dilution threshold to predict post-vaccination boost and, if numbers are sufficient, for viral load as well.

Neutralization titer AUC ROC. While 90% neutralization (NT_{90}) is the most widely used NT percentage for evaluating YFV vaccinee neutralizing Ab titers, there are no data-based justifications for the selection of 90% neutralization in the assay. Alternatives include 50% neutralization, and NT_{50} is commonly used in the DENV field, less frequently in YFV field (*e.g.* [22,32]). As with NT_{90}, no data support NT_{50} as the best evaluation point for NT assays. However, Salje *et al.*[76] analyzed of over 2,000 repeated DENV PRNT done at WRAIR and found that 75% (NT_{75}) was the optimal evaluation point for DENV NT assays based on square bias, variance,

and mean square error of NT estimates compared over a range of NT_{40}-NT_{90}. In Aim 3, we evaluate the clinically meaningful outcome of sterile protection from live attenuated virus challenge. Here we will use AUC ROC analysis to evaluate alternate NT evaluation points (NT_{75} *etc.*) and identify the NT evaluation point that *best discriminates* (sensitive and specific) the NT protective threshold against boost and/or viral load. Using this clinically meaningful NT evaluation point we will re-assess our primary outcome in Aim 3 which is based on an NT_{90} threshold of >1:40 and similarly re-evaluate NT threshold-based tests in Aims 1 and 2.

13.2.4 Safety Data

Safety-related outcomes (Section 11) will be monitored throughout the study period. All AEs occurring on or after YF vaccination will be summarized by body system. If a subject experiences more than one AE, the subject will be counted only once for that AE at the greatest intensity or relationship to YF vaccination. Adverse events will also be summarized according to seriousness, severity, and relationship to the vaccination.

13.2.5 Safety Review

Analyses of safety data will be conducted by the ISM after the first three subjects and at two month interim time points during the study.

Additional safety reviews will occur whenever the stopping rules are met as specified in Section 7.3.

13.3 Sample Size

To execute this study we will work with Dr. Naleway at KPNW to identify previously vaccinated subjects 8 or more years post vaccination. KNPNW vaccinates ~400 individuals/year aged 20-49 years old. Assuming we contact 400 subjects/year with ~4% uptake, we expect to recruit seventeen subjects/year for 2 years for a total of 34 subjects over the study period. Subjects will be recruited pre-vaccination and followed for 28 days post-vaccination with blood draws on days 01 2, 4, 6, 8, 10, 12 ,14 and 28. We assume little to no loss-to-follow up as this study will be conducted over only 65 days, at the end of study Year 5 we expect we will have at least 32 subjects to fully evaluate.

Boost Power calculations. Based the combined studies of Reinhardt[27], Hepburn[31] and Kongsgaard[32], we will assume *a priori* a sterilizing threshold of NT_{90} >1:40. Hepburn provides a robust estimate of 50% (500/1029) of subjects with $NT_{80} \leq$1:40 at 10 years post-vaccination[31], with the caveat that some of these subjects may have been boosted previously. Assuming that 10% of 16 subjects with titers >1:40 boost, and 75% of 16 subjects with titers $\leq$1:40 boost, against the null that 50% will boost each in group, the study has power=0.85 (χ^2=12.28, DF=3, α=0.05, 32 subjects) to detect a difference in boost between two groups divided at an NT_{90} threshold of $\geq$1:40. The study retains power >0.80 with as few as 22 subjects under the same assumptions, and 20 subjects will have power=0.76. There are no prior data available to estimate the proportions of subjects who will develop a detectable viral load on 17D re-vaccination, and we consider evaluation for viral load exploratory but extremely important for the field. We assume that viral load is most likely to be detected in re-vaccinated subjects with $NT_{90} \leq$1:40, or 16 (50%) subjects, and we contend that the null hypothesis is that no more than 1 in 100 will have a detectable viral load as this is consistent with reasoning behind CDC and WHO recommendations for once-in-a-lifetime vaccination. Under these assumptions, the study has power=0.80 (test of 1 proportion[75]) to detect a viral load in at least 1 in 10 subjects.

AUC ROC power calculations. Assuming 18 of 32 subjects boost, the study will have a power =0.80 (α=0.05)[66] to detect AUC ≥0.77 (fair to good range), similar to the AUC=0.740 reported by Kongsgaard *et al.*[32] for the same analysis with 28 subjects. In order for viral load to be informative at the same level, at least 13 subjects would need to have detectable viral loads.

14 HUMAN SUBJECTS PROTECTION

14.1 Discontinuation of Study

The OHSU IRB reserve the right to discontinue the study at any time for administrative reasons. Should either party discontinue the study prior to completion, the subjects already enrolled in the study will be allowed to complete the study, if medically appropriate. In such an event, each enrolled subject will be followed through the period outlined in the study. Investigators will be reimbursed for reasonable expenses incurred to the date of discontinuation on the basis of completed subjects.

14.2 Ethics

14.2.1 Good Clinical Practice and Institutional Review Board Review

Compliance with GCP guidelines for the conduct and monitoring of this clinical study will occur through observation of the ethical and regulatory requirements presented in ICH E6, Good Clinical Practice: Consolidated Guideline. By signing this protocol, the investigator agrees to adhere to these requirements. The study (protocol, informed consent, advertisements, product insert, subject information sheets, and investigator CV and credentials) must be reviewed and approved by the IRB. Changes to study materials (protocol, informed consent and advertisements) must be approved by the local IRB.

14.2.2 Informed Consent

Informed consent is an ongoing process that includes the signing of an informed consent form. Subjects are required to sign an informed consent form prior to being screened, and before undergoing any study procedures or assessments. When substantial modifications are made to the informed consent form, the local IRB may require that all subjects currently enrolled in the study be re-consented. Subjects will be provided with a copy of the signed informed consent form and printed materials that explain the purpose of the study, the medication(s) used in the study, procedures, and assessments. Subjects will also be provided with the telephone numbers of the investigator and qualified personnel who can assist with their questions and concerns.

14.3 Study Risks

14.3.1 Vaccine Risks

YFV-17D is Food and Drug Administration (FDA)-approved in the US for both primary vaccination in persons aged >6 months traveling to YFV endemic regions and booster vaccination in subjects considered high risk for YFV exposure. 17D has a strong record of safety and tolerability dating back to its introduction in 1936. Safety data suggest that booster vaccination as at least 10-fold lower risk of a vaccine related severe adverse event (SAE) compared to primary vaccination. The CDC reports data[74] from observational studies that include 333 million vaccine doses, with 1,255 SAEs overall; vaccination type (primary vs boost)

was reported for 201 SAEs, with 14 (7%) of reported SAEs occurring on booster vaccination. The most serious and potentially life-threatening AE related to this vaccine are neurotropic (YEL-AND) and viscerotropic disease (YEL-AVD). CDC reports YEL-AND observational data[74] for 462 million vaccine doses distributed with 218 YEL-AND cases: vaccination type was known for 110 of the YEL-AND cases, of which 3 (3%) were booster vaccinations. CDC also reports YEL-AVD observational data for 437 million vaccine doses distributed with 72 YEL-AVD cases;[74] vaccination type was known for 31 of the YEL-AVD cases, of which 1 (3%) was a booster vaccination. AEs are least frequently reported in 20-49 year-old vaccinees.

14.3.2 Blood Draw Risks

Risks associated with drawing blood from the arm include some pain when the needle is inserted and a small risk of bruising and/or infection at the place where the needle enters the arm. Some people may experience lightheadedness, nausea, and/or fainting.

14.4 Study Benefits

The proposed studies are not expected to be of direct benefit to study subjects. However, their participation in these studies may further understanding of the mechanisms underlying immunity to yellow fever virus.

14.5 Confidentiality

Demographic data (age, sex) arbovirus exposure history will be obtained by the study coordinator using screening and intake questionnaires developed by the PI and study coordinator. The study coordinator and PI will have access to individually identified private information about human subjects. All subject data collected as part of this study are subject the Health Information Portability and Accountability Act (HIPAA).

14.6 Disclosure of Data

The investigator, his or her staff and associates, and the appropriate regulatory agencies may use the information included in this protocol as necessary for the conduct of the study and the safety of subjects.

14.7 Publication of Research Findings

Manuscript(s) and abstract(s) prepared from the data collected during this trial will be prepared by the study investigators. Results of the study trial will be published without restriction.

15 SUBJECT COMPENSATION

Subjects will be compensated $50 for each completed visit including the screening visit.

The total subject stipend for completing the study will be $500.

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