Study Protocol

Human Immune Responses to Yellow Fever Vaccination

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Human Immune Responses to Yellow Fever Vaccination

(YF Immune Response)

Principal Investigator: Srilatha Edupuganti, MD, MPH
[redacted]
Hope Clinic of Emory University
Decatur, GA 30030
[redacted]

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Co-Investigators at Emory Vaccine Center: Rafi Ahmed, PhD

Donald McGuire, PhD

Sakshi Malik, PhD

External Laboratory Collaborators: Shane Crotty, PhD
Associate Professor, Division of Vaccine Discovery
La Jolla Institute for Allergy & Immunology (LIAI)

Bali Pulendran, PhD
Professor of Microbiology and Immunology
Stanford University

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PROTOCOL SUMMARY

Title: YFV Immune Responses: Human Immune responses to Yellow Fever Vaccination

Population: Healthy males and females between 18 to 45 years of age

Number of sites: 1

Study Duration: 5 years

Number of subjects: 250 adults, ages 18-45. We may need to screen up to 1000 adults to enroll a total of 250 participants due to screen failures)

Subject Participation Duration: 12 months

Description of Intervention: YF-17D (YF-VAX®;Yellow Fever Vaccine)

Objectives:

Primary:

1. Isolation and characterization of yellow fever vaccine-specific adaptive immune responses: characterize the magnitude and quality of yellow fever virus-specific T cell responses, antibody secreting cells and memory B cells.
2. Determine the signatures of innate immune responses: cytokines, chemokines, dendritic cells and microarray analyses on peripheral blood mononuclear cells.

Secondary:

1. Compare the magnitude and kinetics of T and B cell responses, antibody secreting cells and memory B cells at various timepoints.
2. Isolation and characterization, and phenotypic analysis of Epstein-Barr virus, cytomegalovirus and yellow fever virus-specific CD8 T cells.

Description of Study Design:

Healthy adults (18-45 years of age) with no previous vaccination history for flaviviruses and no contraindications will be vaccinated using YF-17D. Serial blood specimens will be collected from participants up to 1 year after YF-17D administration. Peripheral blood mononuclear cell isolation and sorting of yellow fever virus tetramer-specific population will be performed. Subsets of participants will undergo leukapheresis and lymph node fine needle aspirates.

1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background Information

Yellow fever (YF) is a viral disease that is transmitted to humans through the bite of an infected mosquito. Yellow fever is a life-threatening infection that can result in hepatitis, renal failure and coagulation abnormalities, and in severe cases, death[1]. Yellow fever was a major public health threat in the colonial United States in the 18th and 19th centuries.

Yellow fever is endemic in over 40 countries, and approximately 125 countries require proof of vaccination for entry by travelers at risk. An estimated 200,000 cases of yellow fever occur annually in South America and Africa, making it an important vaccine-preventable disease among travelers to endemic areas[2]. Yellow fever can be prevented by vaccination with the Yellow Fever Vaccine, YF-VAX® (made from the 17D strain of YFV). Currently, the Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) recommend the Yellow Fever Vaccine for people aged 9 months to 59 years who are traveling to or living in a yellow fever endemic area[2].

Many flaviviruses, including yellow fever virus (YFV), dengue virus (DEN), West Nile virus, Japanese encephalitis virus and tick-borne encephalitis (TBE) virus are important human pathogens. Many flaviviruses are transmitted by hematophagous arthropods (mosquitoes and ticks). Indeed, dengue, yellow fever, the TBE virus complex and Japanese encephalitis globally represent the most important mosquito-borne diseases causing extensive morbidity and mortality around the world[1]. Even within the United States (US), the dramatic spread of West Nile virus infection over the past few years has provided both the public and public health authorities with a vivid example of the present (and potential future) threats posed by emerging zoonotic infections.

A very interesting, but unexplained aspect of flavivirus biology is how infection with different members of the same virus family can lead to such diverse types of host-virus interactions and variable disease outcomes. For example, YFV infection can be fatal, but if the infected host survives, long-term protective immunity is seen[1, 3, 4]. Alternatively, DEN causes an acute infection with associated acute disease manifestations. However, more severe disease outcomes are observed following secondary infection with a distinct serologic type of DEN[1, 3]. Understanding why the human immune system can successfully contain one flavivirus infection, but not another is both a fascinating scientific enigma in human immunology and a topic with substantial practical importance to public health. Given the great global public health threats posed by epidemic and emerging flavivirus infections, and the need to define the biological basis of successful induction and maintenance of protective immunity by vaccination, elucidation of the immunologic mechanisms underlying the generation and maintenance of protective immunity to the Yellow Fever Vaccine should be extremely useful. Furthermore, definition of the attributes of such a highly effective vaccine should help expedite the development and evaluation of new and/or improved vaccines to prevent important prevalent and emerging infectious diseases.

YF-17D is considered to be one of the safest and most effective viral vaccines ever developed. This vaccine is known to stimulate broad-spectrum immune responses, including cytotoxic T cells, and Th1 and Th2 responses, as well as neutralizing antibody titers that can persist for up to 30 years, after a single vaccination[5, 6]. Despite the great success of this empiric vaccine,

there has been relatively little understanding of the mechanisms by which YF-17D induces such robust protective immune responses. In our past and current U19 grant we have begun to elucidate the innate and adaptive signatures induced by YF-17D in healthy adults between the ages of 18 and 45[7, 8] and T cell memory responses[9-12].

We hope to apply the best contemporary methods in immunology, genomics, and proteomics to characterize in detail a successful immune response to YF-17D. This characterization should identify new immunologic predictors that could serve as surrogates for future vaccine efficacy studies. In addition, these findings could guide development of a safer yellow fever vaccine (or the derivation of safer alternative vaccination regimens using the currently available vaccine).

The goal of this study is to use the live attenuated Yellow Fever Vaccine (YF-VAX®, Sanofi-Pasteur) as a safe and effective model for viral infection to understand human immune response to viral antigens. Yellow fever virus vaccine is the viral infection model that we have chosen for the following reasons:

- The yellow fever live, attenuated 17D vaccine strain is one of the most efficacious vaccines available and has been in use since the 1930s.
- Since most of the US population is not exposed to YFV, immunization of adult participants with the 17D vaccine strain allows for examination of the innate immunity and the naïve T and B cell response in humans during primary infection and the subsequent development of memory T cells after resolution of the primary infection.
- YF-17D vaccination induces long-term immunity that lasts for decades. By studying how the human immune system responds to an effective vaccine such as the Yellow Fever Vaccine, we hope to learn more about the normal functioning of the immune system so that it might be possible to design new, more effective vaccines to prevent important infectious diseases. Therefore, this vaccine can serve as an ideal model to help us decipher the properties that make a vaccine effective.
- YF-17D vaccination leads to limited infection without causing the disease.

1.2 Rationale for including non-travelers and travelers

The Yellow Fever Vaccine (YF-17D; YF-VAX®) is a live, attenuated vaccine that has been used for prevention of yellow fever since 1937 with over 500 million doses administered[2]. There is no treatment available for yellow fever and the only yellow fever vaccine currently licensed for use in the US is YF-VAX®, manufactured by Sanofi Pasteur (Swiftwater, Pennsylvania). According to the Advisory Committee on Immunization Practices (ACIP) guidelines, the Yellow Fever Vaccine is recommended for persons who are aged 9 months and older who are traveling to or living in areas at risk for YFV activity or traveling to a country with an entry requirement for vaccination. In some cases, people younger than 9 months who are at increased risk may receive the yellow fever vaccine. Laboratory personnel who might be exposed to yellow fever virus or vaccine virus may also need YF-VAX®[13]. We plan to enroll adults between 18 to 45 years of age, who are traveling to yellow fever endemic areas, laboratory workers and also those who don't have imminent travel plans.

The Yellow Fever Vaccine is a safe and effective vaccine. Adverse events (AEs) seen with YF-17D are typically mild and may include headache, myalgias, low-grade fever and discomfort at the injection site. These mild AEs occur in about 25% of vaccinees[13]. There have been rare reports of severe AEs. Specifically, the yellow fever vaccine associated neurotropic disease

(YF-AND), and yellow fever vaccine-associated viscerotropic disease (YF-AVD). YF-AND may include post-vaccinal encephalitis, Guillain–Barré syndrome, and autoimmune disease with central or peripheral nervous system involvement. YF-AVD is a disease that clinically resembles naturally acquired infection and may include a spectrum of disease severity, from moderate illness with focal organ dysfunction to severe disease with overt multiple organ system failure and death. The risk factors for YF-AVD are advanced age and thymus dysfunction[2, 5, 6, 13].

AEs seen with administration of the Yellow Fever Vaccine are noted on the Yellow Fever Vaccine Information Sheet (VIS) published by the CDC. All participants will be required to read the most current VIS prior to being vaccinated (https://www.cdc.gov/vaccines/hcp/vis/vis-statements/yf.html). A review of AEs reported to the U.S. Vaccine Adverse Event Reporting System (VAERS) from 2000 to 2006 was published by the CDC[2]. It showed that the overall reporting rate (for all ages) for anaphylaxis was 1.8 per 100,000 doses. YF-AND was 0.8 per 100,000 doses and YF-AVD was 0.4 per 100,000 doses. The reporting rates were higher for vaccinees aged ≥ 60 years – 1.8 and 1.4 per 100,000 doses for YF-AND and YF-AVD, respectively. In general, all AEs were higher in males than females. Based on the above data, and discussions with the Yellow Fever Vaccine experts at the CDC, we feel that the overall risk of a serious adverse event (SAE) for participants we plan to recruit between 18 to 45 years old will be low (0. 4 to 0.8 cases per 100,000 doses).

This study plans to recruit both travelers and non-travelers to yellow fever endemic areas for participation. International travel has been severely restricted in the US due to the COVID-19 pandemic which began in January 2020 and remains ongoing, significantly shrinking the pool of potential traveler participants. We are restricting the maximum age of our participants to 45 years (age cut off by the CDC is 59 years) and we plan to select healthy adults who meet the inclusion/exclusion criteria to minimize any potential yellow fever vaccine-related SAEs (see section 4). To meet the planned enrollment goals of this study (funded via the NIH U19 grant mechanism), we need to be inclusive of both travelers and non-travelers. It is also worth noting that colleagues at other universities who conduct similar studies—since the Yellow Fever Vaccine is a model vaccine—use the approach of including non-travelers. Much of the recruitment will still focus on travelers since they will have the greatest interest in participation.

1.3 Scientific Rationale

To better understand the innate and adaptive immune differentiation of CD8 T cells in response to a virus, we have used the YF-17D human model previously. In these studies, we:

1. Have identified a combination of surrogate markers that identifies CD8 effectors[7] and a YFV-specific CD8 T cell epitope in HLA-A2 specific individuals and characterized it extensively[8].
2. Plan to profile YFV-specific CD8 T cells at different stages of the response post-vaccination. We plan to identify genes that are differentially expressed in these cells and analyze how the pattern of gene expression changes as cells transit from effector and memory cell stages. The surrogate markers and the immunodominant epitope thus far identified will enable physical separation of this cell population. We also plan to profile these cells using microarray technology[8]. We plan to determine the magnitude and quality of YFV-specific T cell responses and the genetic signatures of effector CD8 T cells.

In addition, during activation of antigen specific T cells in response to YF-17D, CD8 T cells dramatically alter their expression of CD45 isoforms. However, unlike canonical CD45RO+ CD45RA- memory cells the bulk of the antigen specific CD8 T cells transition to a CCR7- CD45RA+ phenotype similar to the “TEMRA” population that accumulates in some elderly individuals. Unlike the TEMRA cells, these antigen specific cells retain proliferative capacity have the characteristics of effector memory cells. To date, little is known of the regulation of CD45 isoform expression nor its role in phenotype of effector and memory CD8 T cells. Better understanding of the role and regulation of this abundant phosphatase could allow for improved understanding of the requirement to induce effective long term CD8 memory in response to vaccine change.

3. Plan to determine the output of naïve T cells from thymus and determine the telomere length in T cells.
4. Plan to study the antibody secreting cells and memory B cell responses post-vaccination.
5. Plan to study the epigenetics, specifically, the methylation and demethylation patterns of yellow fever specific CD8 T cells as they move from naïve to effector to memory cells after vaccination. To accomplish this goal, we plan to perform leukapheresis of participants who are positive for the HLA-A202 allele.
6. Plan to study the draining axillary lymph node cells for germinal center reactivities after vaccination, in particular CD4+ T follicular cell, and their functions.
7. Assays proposed in Dr. Crotty’s lab in La Jolla, CA:

- Yellow fever-specific T-B cell co-culture assay in vitro to measure the B cell helper ability of the YFV-specific CD4 T cells
- YFV-specific T cell proliferation in vitro
- YFV specific cytokine/chemokine production in vitro
- Phenotypical characterization of YFV-specific CD4 T cells by Flow Cytometry. This analysis also requires HLA typing of the donors.

1.4 Potential Risks and Benefits

1.4.1 Potential Risks

YF-VAX® is an FDA approved vaccine that is routinely recommended for persons 9 months of age or older who are traveling or living in a country where yellow fever is endemic. However, there are some risks associated with YF-VAX®. Mild reactions include soreness, redness, and swelling at the injection site, mild headache, muscle aches and mild fever and in up to 25% of the vaccines 5 to 10 days after vaccination. Severe reactions to the vaccine have been reported but are extremely rare. (Please refer to the Vaccine Information Sheet and Section 1.2).

1.4.2 Known Potential Benefits

Currently, CDC and WHO recommend vaccination for persons 9 months of age or older who are traveling to or living in a yellow fever endemic area[13]. YF-VAX® is highly efficacious and is protective from yellow fever virus infection, which is potentially life-threatening.

2 OBJECTIVES

2.1 Primary:

1. Isolation and characterization of yellow fever vaccine-specific adaptive immune responses: characterize the magnitude and quality of yellow fever virus-specific T cell responses, antibody secreting cells and memory B cells.
2. Determine the signatures of innate immune responses: cytokines, chemokines, dendritic cells and microarray analyses on peripheral blood mononuclear cells.

2.2 Secondary:

1. Compare the magnitude and kinetics of T and B cell responses, antibody secreting cells and memory B cells at various timepoints.
2. Isolation and characterization, and phenotypic analysis of Epstein-Barr virus, cytomegalovirus and yellow fever virus-specific CD8 T cells.

3 STUDY DESIGN

This is an observational study of immune responses to YF-17D vaccination in healthy adults. The study will be conducted at the Hope Clinic of Emory Vaccine Center.

A total of 250 healthy participants (18-45 years of age) will be enrolled into four study arms: A, B, C & D. Arm enrollment is determined by Human Leukocyte Antigen (HLA) type, current needs of the lab and/or willingness to participate in sampling procedures.

All participants will receive YF-17D on Day 0 at the FDA-approved dose and route of administration. Post-vaccination procedures are determined by arm assignment.

The study arms are as follows:

- Arm A (n = 200): HLA-A202^{+}– post-vaccination blood draws
- Arm B (n = 10): HLA-A202^{+} – post-vaccination blood draws and leukapheresis
- Arm C (n = 20): HLA-A202^{+} – post-vaccination blood draws and fine needle aspirate
- Arm D (n = 20): HLA-A202^{-} – post-vaccination blood draws

In general, follow up visits will occur on Days 3, 7, 14, 21, 28, 90, 180, and 360 post-vaccination.

Please see Study Schema in **Appendix A** for detailed flow chart of study Arms A-D.

4 STUDY ENROLLMENT

4.1 Subject Inclusion Criteria

1. Able to understand and give informed consent
2. Age 18-45 years
3. Participant agrees not to take any live vaccines 30 days before or after (14 days for inactivated, including COVID-19 vaccination) yellow fever vaccination
4. Women of childbearing potential must agree to use effective birth control throughout the duration of the study. A negative urine pregnancy test must be documented prior to vaccination. Participants who have a history of surgical sterilization or post-menopausal status >1 year, are not required to have a pregnancy test.

4.2 Subject Exclusion Criteria

1. Prior receipt of a yellow fever vaccine
2. Lived in a country/area which is endemic for yellow fever
3. Travel to country/area which is endemic for yellow fever. Subject to investigator discretion
4. History of previous yellow fever, West Nile, Dengue, St. Louis encephalitis, Japanese encephalitis vaccination or infection
5. Any history of allergy to eggs, chicken or gelatin or to any previous vaccine
6. A history of a medical condition resulting in impaired immunity (such as HIV infection, cancer, particularly leukemia, lymphoma, use of immunosuppressive or antineoplastic drugs or X-ray treatment). Persons with previous skin cancers or cured non-lymphatic tumors are not excluded from the study
7. History of HIV infection
8. Active Hepatitis B or Hepatitis C infection
9. COVID-19 infection in the last 30 days. Symptoms of COVID-19 must be completely resolved before yellow fever vaccine receipt.
10. History of any chronic medical conditions that are considered progressive (ex, diabetes, heart disease, lung disease, liver disease, kidney disease, gastrointestinal diseases and uncontrolled hypertension). Use of systemic immunosuppressive medications (ex, prednisone) for 2 weeks or more in the past 3 months
11. History of excessive alcohol consumption, drug abuse, psychiatric conditions, social conditions or occupational conditions that in the opinion of the investigator would preclude compliance with the trial
12. Thymus gland problems (such as myasthenia gravis, DiGeorge syndrome, thymoma) or removal of thymus gland or history of autoimmune disorder

13. Recipient of a blood products or immune globulin product within 42 days of the vaccination visit. Participants who received COVID mAbs for treatment are not excluded
14. Pregnant women and nursing mothers or women who are planning to become pregnant for the duration of the study
15. Any condition in the opinion of the investigator that would interfere with the proper conduct of the trial

4.3 Randomization Procedures

There is no randomization in this study. The arm to which a subject is assigned to is determined by their HLA type, current lab needs and/or willingness to participate in sampling procedures.

5 STUDY INTERVENTION

5.1 Study Product Description

YF-VAX® is manufactured by Sanofi Pasteur as one-dose vials will be purchased from the manufacturer. Vaccine will be stored at the Emory Investigational Drug service between 2 to 8°C per the manufacturer's instructions.

5.2 Dosage, Preparation and Administration of Study Product

YF-VAX® will be stored, prepared and dispensed from the Hope Clinic Investigational Drug Services (IDS) – Hope Clinic Satellite Location.

The vaccine will be administered by a nurse at the Hope Clinic with a 5/8th to ¾ inch long needle via subcutaneous injection.

5.3 Accountability Procedures for the Study Vaccine

The IDS pharmacist will maintain accurate drug accountability logs which will be kept at the IDS pharmacy until the completion of the study. Upon completion of the study, a final drug accountability will be done.

6 STUDY PROCEDURES

6.1 Screening Visit

When a potential participant presents for the screening visit, the informed consent will be reviewed, and all questions answered. Once the subject signs the informed consent, a study participant number will be assigned. Eligibility will then be determined for each participant based primarily on information gathered during the screening visit. Procedures conducted during the screening visit may include:

- Collection of medical history
- Collection of current medications
- Physical exam
- Vital signs
- Blood sample collection
- HLA typing (if not previously known)

At this visit, if the participant reports having received a vaccination against COVID-19 in the past 14 days or had a COVID-19 infection in the past 30 days, the enrollment visit will be delayed. All symptoms following a COVID-19 infection must be resolved before the enrollment visit.

6.2 Enrollment/Vaccination Visit (Day 0)

This visit will serve as an enrollment and vaccination visit for all participants. This visit may occur on the same day as the Screening Visit if the participant's HLA type is known prior to Screening. At this visit, the following activities may be performed:

- Physical exam
- Vital signs
- Collection of current medications
- Blood sample collection
- Urine pregnancy test (for women of childbearing potential)
- AE collection (any AE present prior to vaccination will be considered medical history)
- Vaccine administration
- Reactogenicity symptom diary distribution

Prior to vaccine administration, participants will be asked to read the Vaccine Information Sheet for the Yellow Fever Vaccine per CDC guidelines. After vaccination, participants will be observed for a minimum of 20 minutes for any immediate hypersensitivity reactions and will be given the International Certificate of Vaccination.

6.3 Follow-up Visits

The follow-up schedule that a participant follows will be dictated their Arm assignment and current needs of the lab (designated as optional visits). Please see Tables 1 and 2 for a full Schedule of Activities (SOA). The following activities may take place at follow-up visits.

- Review of current medications
- Blood Sample Collection
- Collection of Solicited AEs

6.4 Leukapheresis Procedure

Participants in Arm B will undergo this procedure on either Day 14 or 28. Collection of CD8 T Cells via leukapheresis will be performed in accordance with the standard practices of the Emory University Hospital Hemapheresis Center. Typically, in this procedure, approximately 12 liters of blood will be processed over about 3 to 4 hours using peripheral veins for venous access. Samples will be sent from the Hemapheresis Center to the Emory Vaccine Center Labs.

6.5 Fine Needle Aspirate Procedure

Participants in Arm C will undergo this procedure on either Day 14 or 28. Tissue sampling of an axillary lymph node will be carried out percutaneously by fine needle aspiration (FNA). This procedure will be performed in accordance with the standard practices of the Winship Cancer Center by Emory Radiology Department providers. The procedure involves tissue retrieval with a needle under sonographic guidance. Approximately 2-4 passes will be made to retrieve cytologic material. Samples will be sent from Winship to the Emory Vaccine Center Labs.

6.6 Schedule of Activities

Table 1. Schedule of Activities – Arms A (Standard), B (Leukapheresis), C (FNA) & D (HLA-A202⁻)

Visit	Screen	Day 0/ Enrollment	Day 3[1]	Day 7[1]	Pre-Leuka[2]	Day 14	Day 21[1]	Day 28	Day 90	Day 180	Day 360
Window (Days)	–	–	–1	± 1	–2 prior to leuka	± 1	± 5	± 5	± 28	± 30	± 90
Informed Consent	X										
Medical History	X	X[3]									
Current Medications	X	X[3]	X	X		X	X	X	X	X	X
Physical Exam		X									
Urine Pregnancy Test		X			X	X[3]		X[3]			
Vitals	X	X[3]									
Confirm Eligibility	X										
Vaccination		X									
Symptom Diary		Distribute		Collect							
Leukapheresis[2,4]						X		X			
FNA[4, 5]						X		X			
AE Collection			X	X		X	X	X			
Blood Sample (Max Volume)[6]	20 ml	20 ml	24 ml	24 ml	5 ml	74 ml[7]	74 ml	74 ml[7]	106 ml	106 ml	106 ml

[1]Visit may be optional based on current needs of the lab.
[2]Only applicable for Arm B participants.
[3]If needed.
[4]Only if undergoing FNA procedure that day.
[5]Participants have procedure on either Day 14 OR 28 – not both. Specific day depends on the current needs of the lab. Day 14 window: -2/+4 days; Day 28 window: ± 4 days.
[6]Only applicable for Arm C participants.
[7]Blood volume will be adjusted as per the laboratory needs at that point in time, no more than the maximum allowable.
[8]Blood sample not collected on the same day a participant undergoes leukapheresis.

7 SAFETY ASSESSMENT

7.1 Adverse Events and Serious Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it will be recorded as an AE. The start and stop date of each reported AE will be recorded on the appropriate CRF. AEs characterized as intermittent require documentation of onset and duration of each episode.

AEs occurring during the trial-collection and reporting period will be documented appropriately regardless of relationship to study product. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. AEs will be followed through resolution.

A summary report of all AEs will be reviewed at regularly scheduled quarterly meetings by the study team.

Serious Adverse Event (SAE): An AE or suspected adverse reaction is considered “serious” if, in the view of either the Principal Investigator (PI) or appropriate sub-investigator, it results in any of the following outcomes:

- Death,
- A life-threatening AE*,
- Inpatient hospitalization or prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

* Life-threatening AE. An AE is considered “life-threatening” if, in the view of either the PI, sub-investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

7.2 Collection of Adverse Events

AEs and SAEs will be collected via a participant-completed reactogenicity symptom diary, participant interviews and/or direct evaluation. Participants may also reach out to study staff to report AEs. If needed, the subject may be asked to return to the Hope Clinic for additional safety procedures to further evaluate an AE or SAE.

- Solicited injection site and systemic reactogenicity AEs will be collected from the time of study vaccination through 7 days after study vaccination.
- Unsolicited non-serious AEs will be collected from the time of study vaccination through approximately 30 days after study vaccination.
- SAEs (regardless of relation to study product) will be collected for 12 months post-vaccination (or last study visit, whichever occurs first).

7.3 Assessment of Severity of Adverse Events

AEs must be assessed for severity and relationship to study product (see definitions below).

AEs will be assessed by a licensed study PI or sub-investigator using the FDA CBER Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers enrolled in Preventive Vaccine Clinical Trials (Sept 2007) (see Appendix B) except:

- Unintentional weight loss is required to be reported as an AE only if it is considered to be potentially deleterious to the participant's health
- Injection Site Erythema or Redness and Injection Site Induration or Swelling will not consider interference with usual social and functional activities such that:
  - Grade 1 is: 2.5 to < 5 cm in diameter OR 6.25 to < 25 cm2 surface area;
  - Grade 2 is: ≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm2 surface area;
  - Grade 3 is: ≥ 10 cm in diameter OR ≥ 100 cm2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage;
  - Grade 4 is: Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)

Additionally, chills and arthralgia will be graded according to the following criteria:

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	N/A
Arthralgia	Joint pain causing no or minimal Interference with usual social & functional activities	Joint pain causing greater than minimal interference with usual social & functional activities	Joint pain causing inability to perform usual and & functional activities	Disabling joint pain causing inability to perform basic self-care functions

For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

- Mild (Grade 1): Events require minimal or no treatment and do not interfere with the subject's daily activities.
- Moderate (Grade 2): Events result in a low level of inconvenience or concern with therapeutic measures. Moderate events may cause some interference with functioning and daily activities.
- Severe (Grade 3): Events interrupt the subject's daily activities and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- Potentially life threatening (Grade 4): ER visit or hospitalization

7.4 Relationship to Study Intervention

The study products that must be considered in determining relationships of AEs are:

- YF-VAX®

The licensed study physician's assessment of an AE's relationship to study product is part of the documentation process, but it is not a factor in determining what is or is not reported in this trial. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

Study investigators will assess all AEs as being related or unrelated to the vaccination.

7.4.1 Assigning AE Relationship to Study Product

An inability to exclude or "rule out" a relationship to the study product is not, in and of itself, sufficient to deem an AE "related."

Use the following definitions when reporting attribution to study product:

- Choose "Related" if there is a reasonable possibility* that the AE may be related to the study product. This choice suggests that the AE is more likely to be related than not related to the study product(s).

- Choose “Unrelated” if there is not a reasonable possibility* that the AE is related to the study agent. This choice suggests that the AE is more likely to be related to another cause rather than the study product(s).

*The U.S. Federal Drug Administration has published a final rule amending the IND

safety reporting requirements under 21 CFR part 312.

- The final rule set a higher standard for reasonable possibility of relationship of study product to an adverse event.
- It defined that, for the purposes of IND safety reporting by the study sponsor, “reasonable possibility” means there is evidence to suggest a causal relationship between the study product and the adverse event.

7.5 Reporting Adverse Events

Because YF-VAX® is an FDA-approved vaccine, AEs that meet the following criteria will be reported to the Vaccine Adverse Events Reporting System (VAERS):

- Local and Systemic Reactogenicity AEs ≥ Grade 3
- Related AEs ≥ Grade 3
- Related SAEs

AEs and SAEs will be reported to the Emory IRB per their guidelines.

8 LABORATORY

8.1 Laboratory Assays or Procedures

The blood will be processed to isolate peripheral blood mononuclear cells (PBMCs) and serum. Based on laboratory supply availability, either Cell Preparation Tube (CPT) or Acid Citrate Dextrose (ACD) will be used.

Plasma from all time points will be separated from whole blood and will be frozen at -80^{o}C. Peripheral blood mononuclear cells will be isolated on all study days. The 17D vaccine-induced viremia will be determined by TaqMan real-time PCR assay of the plasma (Applied Biosystems, Foster City, CA). The lower limit of detection for this assay is 25copies/ml and will be performed in the Molecular Virology Core of the Emory Vaccine Center.

The following assays will be performed for innate immunity:

1. Serum analyses of cytokines and chemokines that regulate adaptive immunity.
2. Phenotype, activation status and frequencies of dendritic cell (DC) subsets and DC precursors in the blood
3. Microarray analyses.

The following assays will be performed for the adaptive immunity:

1. Magnitude of the YFV-specific effector T cell response

2. Quality of the YFV-specific T cell response by determining the expression of various markers of cytolysis (granzymeB and perforin), homing (CD62L, CCR7, a4b7, CLA), cytokine receptors (CD127, CD122), costimulation (CD27, CD28), inhibition (PD-1), adhesion (CD11a) and survival (Bcl-2) will be analyzed using 11-parameter flow cytometry.
3. Output of naïve T cells from the thymus using the TREC assay
4. YFV-specific serological responses
5. Analysis of the YFV-specific ASC variable gene repertoire
6. Gene profiling of the YFV vaccine-induced antibody secreting cells (ASC)
7. Yellow Fever-specific T-B co-culture assay in vitro to measure the B cell helper ability of the YFV-specific CD4 T cells
8. YFV-specific T cell proliferation in vitro
9. YFV-specific cytokine/chemokine production in vitro
10. Phenotypical characterization of YFV-specific CD4 T cells by Flow Cytometry. This analysis requires also an HLA typing assay of the donors.
11. YFV-specific CD4 and CD8 T cells in axillary lymph node aspirates

The PBMCs will be stained using a combination of surface markers to identify activated or YFV tetramer specific CD8 T cells. The CD8 T cell populations of interest will be isolated using flow cytometry-based sorting. The sorted populations will be processed for RNA isolation and microarray analysis performed using affymetrix gene arrays. Gene expression values will be obtained using robust multiarray analysis (RMA).

A small part of the PBMCs will be stored for validation of expression of certain signature genes by RT-PCR and for in vitro functional assays. Plasma will be stored and later tested for antibodies against yellow fever virus. At all visits PBMCs are harvested and the following tests will be done to analyze the T cell responses:

- FACS analysis for activation markers (such as CD69, CD38, CD45, CD62L, etc.) on CD8 and CD4 T cells and B cells.
- CFSE labeling of PBMCs, stimulation with antigenic peptides and evaluation of proliferative responses as seen by CFSE dilution in flow cytometric analysis.

8.2 Specimen Preparation, Handling, and Shipping

The blood tubes will be labeled at the Hope Clinic with a unique identifier and will be transported to the Emory Vaccine Center via courier service in appropriate transport containers.

9 STATISTICAL CONSIDERATIONS

This is an exploratory non-placebo-controlled analysis of variation in the cellular and antibody specific immune responses generated against YFV. This will provide us with descriptive data to analyze the immune responses. The microarray data generated will be analyzed using Benjamin’s Fisher test.

10 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

All source documents will be kept at the Hope Clinic in a locked cabinet in a locked office. The building is secured by a monitored alarm system.

11 QUALITY CONTROL AND QUALITY ASSURANCE

Per Hope Clinic Standard Operating Procedures (SOPs), a portion of the study charts (approximately 25%) will be audited by the Hope Clinic Quality Management (QM) personnel and all charts will be audited for informed consent compliance.

12 ETHICS/PROTECTION OF HUMAN SUBJECTS

This study will be conducted in compliance with all applicable federal regulations and institutional policies. The study team will get IRB approval of all applicable study documents prior to use. Informed consent will be sought from all participants prior to any study activities occurring.

13 DATA HANDLING AND RECORD KEEPING

Participant data will be maintained at the Hope Clinic in a locked file cabinet. Biological samples and immunological assays data will be labeled with only a unique study code number and maintained in the laboratory of Dr. Rafi Ahmed and Dr. Bali Pulendran. The links to the unique codes will be stored at the Hope Clinic and will not be available to the laboratory personnel.

The study records will be maintained up to ten years after the completion of the study.

14 PUBLICATION POLICY

The PI and/or sub-investigators will participate in manuscript preparation and submission to journals.

15 LITERATURE REFERENCES

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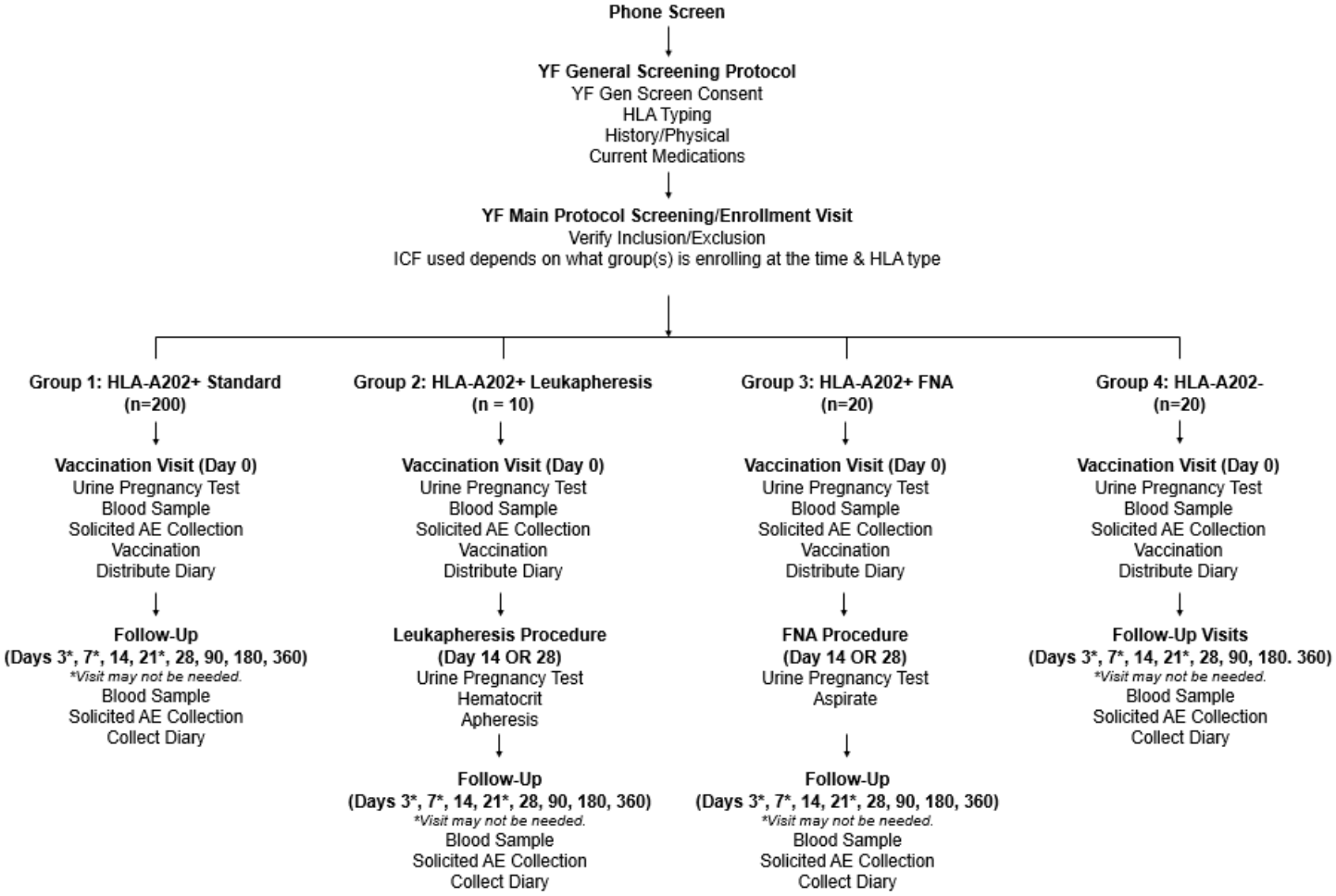
16 APPENDICES

APPENDIX A. Flow chart for Yellow Fever Immune Response Study



APPENDIX B. Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials (FDA, September 2007)

Tables for Clinical Abnormalities

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness *	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling **	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

*In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
**Induration/Swelling should be evaluated and graded using the functional scale

Vital Signs*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C) ** (°F) **	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

*Subject should be at rest for all vital sign measurements.
**Oral temperature; no recent hot or cold beverages or smoking.
*** When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

Systemic Illness	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization