Systems Biology of Inactivated Rabies Vaccine in Healthy Adults With or Without Use of Broad Spectrum Antibiotics.

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PRINCIPAL INVESTIGATOR

Nadine Rouphael, MD
Hope Clinic, 500 Irvin Court, Suite 200
Decatur, GA 30030
Office: 404-712-1435
Cell: 404-822-1411
Pager: 404-686-5500 (PIC 16509)
Fax: 404-499-9727
Email: nroupha@emory.edu

CO- PRINCIPAL INVESTIGATOR

Mary S Newell, MD
1701 Uppergate Drive, Suite C1104
Atlanta, GA 30322
Office: 404-778-4446
Email: mnewll@emory.edu

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INVESTIGATOR SIGNATURE PAGE	
Protocol Systems Biology of Inactivated Rabies Vaccine in Healthy Adults With or Without Use of Broad Spectrum Antibiotics	**Version/Date:** 5.0/ 06/28/2021
	Principal Investigator: Nadine Rouphael, MD
Short Title*:* Responses to Rabies Vaccine in Adults with or without Antibiotics.	
INSTRUCTIONS: The Principal Investigator will print, sign, and date at the indicated location below. A copy should be kept in the investigator's records.	
I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) 45 CFR part 46 and 21 CFR parts 50, 56, and 312 , and the International Conference on Harmonization (ICH) document "Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance" dated April 1996[1] and the spirit of the Declaration of Helsinki, Further, I will conduct the study in keeping with local, legal, and regulatory requirements. As the Principal Investigator, I agree to conduct "Systems Biology of Inactivated Rabies Vaccine in Healthy Adults With or Without Use of Broad Spectrum Antibiotics"**.** I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission by the local IRB. ___NADINE ROUPHAEL, MD_____ Principal Investigator (Print) _____ _____ Principal Investigator (Signature)	

Synopsis

Title	Systems Biology of Inactivated Rabies Vaccine in Healthy Adults With or Without Use of Broad Spectrum Antibiotics.
Short Title	Responses to Rabies Vaccine in Adults with or without Antibiotics
Rationale	Use of antibiotics can significantly impact the microbiota of patients. The status of the intestinal microbiome may impact the immune responses to vaccinations and alter functional protective immunity in humans. Here we hypothesize that the changes in the fecal microbiome induced by antibiotics translates into suboptimal antibody response to rabies vaccine.
Clinical Phase	N/A
Mechanistic Study	Yes
Principal Investigator	**Nadine Rouphael, MD**
Participating Site(s)	**The Hope Clinic of the Emory Vaccine Center** The Hope Clinic is a community-based vaccine research clinic and is the clinical arm of the Emory Vaccine Center at Emory University. Address: 500 Irvin Ct, Suite 200 Decatur, Georgia 30030 **Winship Cancer Institute of Emory University** Address: 1365 Clifton Road NE, Bldg. C Atlanta, Georgia 30322
Accrual Objective	N=5-8 for phase 1 N=24 for phase 2 N=22 for phase 3
Primary Study Objective	To compare antibody titers after vaccination with Rabies vaccine in adults with or without use of antibiotics
Study Design	Single center, mechanistic study with 3 phases in which subjects will be randomized to receive one dose of Rabies vaccine at day 4 of a 5 day course of a specific antibiotic regimen (Group A) or Rabies vaccine alone (Group B) followed by a second dose 28 days later for both groups. The immune responses in the blood and the lymph node will be analyzed. Phase 1 of the study will determine the best strategy for sampling the lymph node (either by fine needle aspiration (FNA) or by core needle

	biopsy); phase 2 of the study will determine if there is a difference in antibody response in the blood in subjects receiving rabies vaccine with or without antibiotics. Phase 3 will compare the difference in immune responses in the blood and lymph node in subjects receiving rabies vaccine with or without antibiotics. Blood samples for immunologic testing will be collected at screening (from D -35 to D -2), on D0 (at vaccination), D1, D3, D7 (+/- 1 day), D10 (-2/+4), D14 (+/- 2 day), D28 (+/- 5 days), D29, D35 (+/- 1 day), D38(-2/+4), D56 (+/- 5 days), D180 (+/- 14 days), D365 (+/-14 days) post vaccination for both groups to study innate and/or adaptive immune responses. Stool samples will be collected in both groups at screening (from D -35 to D -2), on D0 (at vaccination), D1, D3, D7 (+/- 1 day), D10 (-2/+4), D14 (+/- 2 day), D28 (+/- 5 days), D29, D35 (+/- 1 day), D38(-2/+4), D56 (+/- 5 days), D180 (+/- 14 days), D365 (+/-14 days) post vaccination to study the gut microbiome. Antibiotics received by Group A will be started 3 days prior to vaccination (D-3) and continued on day of vaccination (D0) and for one day after vaccination (D1) for a total of 5 days. • Flagyl® 500 mg po tid • Vancocin® 125 mg po qid • Neomycin sulfate ® 500mg po tid The dosage of each antibiotic is taken from their respective package inserts and does not exceed the maximum dose allowed for each antibiotic. The antibiotic regimen is a broad spectrum regimen covering all types of fecal flora (anaerobes, gram positive, and gram negative bacteria); has a good safety record; has poor systemic absorption for part of the regimen (Vancocin® and Neomycin sulfate®); and part of the regimen is used to treat *Clostridium difficile* infections (Vancocin® and Flagyl®). Subjects in Group A are asked to avoid all ethanol and any ethanol-containing drugs while taking antibiotics and for 48h before and after taking the antibiotics. Blood samples for safety laboratory testing (including CBC with differential, creatinine, potassium) will be collected at screening (from D-35 to D -2) and D 7 (+/- 1 day) for both groups (phases 2 and 3). For Group A, stools will be screened for *Clostridium difficile* carriage by PCR at screening (from D-35 to D-2). Lymph node sampling will be done once in phase 1. Lymph node sampling will be done in phase 3 10 days after first and second vaccinations
Study Duration	Approximately 66 months
Primary Endpoints	Comparison of antibody titers at D28 post first vaccination in both groups.
Secondary Endpoints	Frequency, severity, and causality of all adverse events in Group A subjects and all adverse events related to sampling of the lymph node.

Exploratory Endpoints	• Analyses of microbiome at D0, D1, D3, D7, D10, D14, D28, D35, D38, D56, D180, and D365, and comparison to baseline screening in both groups. • Analysis of the repertoire and monoclonal antibodies from plasmablasts in a subset of vaccinees at D7 and D35 in both groups. • Identification of innate immune signatures (traditional immune parameters measurements + array-based gene expression) at D1, D3 and D7 in both groups. • Correlation of innate immune signatures at days D1, D3 and D7 with antibody titers at D28 and D56 in both groups • Description of immune profiling in the blood and the lymph node at different time points.
Inclusion Criteria (Phase 1)	1. Healthy individuals aged 18-49 years. 2. Able to understand and give informed consent.
Exclusion Criteria (Phase 1)	1. Receipt of the following: • Receipt of blood products 3 months prior to procedure. • Receipt of any vaccine 4 weeks prior to procedure. 2. Presence of co-morbidities or immunosuppressive states such as: • Chronic medical problems including (but not limited to) insulin dependent diabetes, severe heart disease (including arrhythmias), severe lung disease, severe liver disease, severe kidney disease, auto immune diseases, and grade 4 hypertension*. • Chronic neurologic conditions including seizure disorder, Parkinson's disease, myasthenia gravis, neuropathy, or history of encephalopathy, meningitis or ototoxicity. • Alcohol abuse, drug abuse, or psychiatric conditions that in the opinion of the investigator would preclude compliance with the trial. • Any history of lymphoma involving axillary nodes or any history of breast cancer. • Impaired immune function or known chronic infections including, but not limited to, known HIV, tuberculosis, hepatitis B or C; organ transplantation (bone marrow, hematopoietic stem cell, or solid organ transplant); immunosuppression due to cancer; current and/or expected receipt of chemotherapy, radiation therapy, steroids** (i.e., more than 20 mg of prednisone given daily or on alternative days for 2 weeks or more in the past 90 days , or high dose inhaled corticosteroids***); and any other immunosuppressive therapies (including anti-TNF therapy), functional or anatomic asplenia, or congenital immunodeficiency. • Pregnancy or breast feeding. 3. Conditions that could affect the safety of the volunteers, such as:

	• History of bleeding disorders or current use of warfarin, aspirin, heparin, nonsteroidal anti-inflammatory drugs (NSAIDs) or other blood thinner/anticoagulant medications in the past week. • Any allergy to lidocaine. 4. Volunteers with any acute illness, including any fever (≥ 100.4 F [≥ 38.0C], regardless of the route) within 3 days prior to vaccination****. 5. Social, occupational, or any other condition that in the opinion of the investigator might interfere with compliance with the study and vaccine evaluation. 6. Subjects who believe they cannot tolerate the procedure without sedation. 7. Bilateral inflammatory process of upper arms in the past 2 weeks. 8. Prior breast or axillary biopsy and/or surgery. **Note:** *Grade 4 hypertension per CTCAE criteria is defined as life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive). **Subjects receiving ≥ 20 mg/day of prednisone or its equivalent daily or on alternate days for more than 2 weeks may enter the study after therapy has been discontinued for more than 3 months. *** Subjects are excluded if on high dose intranasal steroids defined as > 960 mcg/day of beclomethasone dipropionate or equivalent. ****An individual who initially is excluded from study participation based on one or more of the time-limited exclusion criteria (e.g., acute illness, receipt or expected receipt of live or inactivated vaccines) may be reconsidered for enrollment once the condition has resolved as long as the subject continues to meet all other entry criteria.
Inclusion Criteria **(Phases 2 & 3)**	1. Healthy individuals aged 18-49 years. 2. Able to understand and give informed consent. 3. Women of child-bearing potential (not surgically sterile via tubal ligation, bilateral oophorectomy or hysterectomy or who are not postmenopausal for ≥1 year) must agree to practice adequate contraception that may include, but is not limited to, abstinence, monogamous relationship with vasectomized partner, barrier methods such as condoms, diaphragms, spermicides, intrauterine devices, and licensed hormonal methods* for 28 days before and 28 days after rabies vaccination. * Women of child-bearing potential using licensed hormonal methods must also use a second form of contraception.
Exclusion Criteria **(Phases 2 & 3)**	1. Receipt of the following: • Receipt of blood products 3 months prior to vaccination or expected receipt through 12 months after vaccination. • Receipt of any live virus vaccines within 28 days prior to vaccination or expected receipt within 28 days after vaccination.*

	• Receipt of any inactivated vaccine within 14 days or expected receipt within 14 days after vaccination other than study vaccine.* • Receipt of any antibiotic 3 months prior to vaccination or expected receipt 28 days after vaccination. • Receipt of probiotics and prebiotics 3 months prior to vaccination or expected receipt 28 days after vaccination. • Receipt of proton pump inhibitors, H2 receptor blockers, or antacids 3 months prior to vaccination or expected receipt 28 days after vaccination. 2. Presence of co-morbidities or immunosuppressive states such as: • Chronic medical problems including (but not limited to) insulin dependent diabetes, severe heart disease (including arrhythmias), severe lung disease, auto immune diseases, thrombocytopenia and grade 4 hypertension**. • Chronic neurologic conditions including seizure disorder, Parkinson's disease, myasthenia gravis, neuropathy, or history of encephalopathy, meningitis or ototoxicity. • Any history of gastrointestinal disease including (but not only): documented bacterial gastroenteritis or gastroenteritis associated with fever or associated with presence of blood/mucus in stools in the last 3 months; inflammatory bowel disease, and/or gastrointestinal surgery. • Any history of kidney or liver diseases. • Alcohol abuse, drug abuse, or psychiatric conditions that in the opinion of the investigator would preclude compliance with the trial or interpretation of safety or endpoint data. • Any history of lymphoma involving axillary nodes or any history of breast cancer. • Impaired immune function or known chronic infections including, but not limited to, known HIV, tuberculosis, hepatitis B or C; organ transplantation (bone marrow, hematopoietic stem cell, or solid organ transplant); immunosuppression due to cancer; current and/or expected receipt of chemotherapy, radiation therapy, steroids*** (i.e., more than 20 mg of prednisone given daily or on alternative days for 2 weeks or more in the past 90 days , or high dose inhaled corticosteroids****); and any other immunosuppressive therapies (including anti-TNF therapy), functional or anatomic asplenia, or congenital immunodeficiency. • Pregnancy or breast feeding 3. Conditions that could affect the safety of the volunteers, such as: • Severe reactions to prior vaccinations, including anaphylaxis • History of Guillain-Barré syndrome • History of bleeding disorders or current use of warfarin, aspirin, heparin, nonsteroidal anti-inflammatory drugs (NSAIDs) or other

	blood thinner/anticoagulant medications in the past week (for subjects undergoing lymph node sampling) • Use of anticonvulsants • Use of digoxin or other forms of digitalis • Any allergy to any component of the vaccine or lidocaine (for subjects undergoing lymph node sampling) • Allergy to vancomycin, metronidazole or neomycin as well as other aminoglycosides (gentamicin, tobramycin, amikacin, streptomycin) 4. Volunteers with any acute illness, including any fever (≥ 100.4 F [≥ 38.0C], regardless of the route) within 3 days prior to vaccination *. 5. Social, occupational, or any other condition that in the opinion of the investigator might interfere with compliance with the study and vaccine evaluation. 6. Positive *C difficile* testing by PCR at screening or history of *C difficile* infection. 7. Any grade 2 safety lab test results at screening 8. Previously received any rabies vaccine or immunoglobulin prior to study entry. 9. Are at high risk of exposure to rabies: veterinarians, animal handlers, rabies laboratory workers, spelunkers, frequent contact with rabies virus or with possibly rabid animals, international travelers who are likely to come in contact with animals in parts of the world where rabies is common, and rabies biologics production workers. 10. Bilateral inflammatory process of upper arms in the past 2 weeks. 11. Prior breast or axillary biopsy and/or surgery. **Note:** *An individual who initially is excluded from study participation based on one or more of the time-limited exclusion criteria (e.g., acute illness, receipt or expected receipt of live or inactivated vaccines) may be reconsidered for enrollment once the condition has resolved as long as the subject continues to meet all other entry criteria. ** Grade 4 hypertension per CTCAE criteria is defined as Life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive). ***Subjects receiving ≥ 20 mg/day of prednisone or its equivalent daily or on alternate days for more than 2 weeks may enter the study after therapy has been discontinued for more than 3 months. ****Subjects are excluded if on high dose intranasal steroids defined as > 960 mcg/day of beclomethasone dipropionate or equivalent.
Investigational Product(s)/ Intervention(s)	Licensed inactivated rabies vaccine: Imovax® (Sanofi Pasteur, Philadelphia, PA) Lidocaine 1% Antibiotics:

	Flagyl® (Pfizer, New York, NY) Vancocin® (ViroPharma, Exton, PA) Neomycin sulfate® (X-Gen Pharmaceuticals, Big flats, NY)
Study Procedures	Antibiotic administration, draining lymph node sampling (either by fine needle biopsy or core needle biopsy), rabies vaccination, targeted physical examination, urine pregnancy testing, phlebotomy, stool collection, signs/symptom assessment (local and systemic) and vaccination site evaluation.

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GLOSSARY OF ABBREVIATIONS

ACIP	Advisory Committee on Immunization Practices
AADCRC	Asthma and Allergic Diseases Cooperative Research Center
AE	Adverse Event
CFR	Code of Federal Regulations
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
cGCP	Current Good Clinical Practice
GLP	Good Laboratory Practice
ICH	International Conference on Harmonization
IDE	Investigational Device Exemption
IFNg	Interferon gamma
IIV	Inactivated Influenza Vaccine
IL	InterLeukin
IM	IntraMuscular
PO	Per Os (by mouth)
ISM	Independent Safety Monitor
IND	Investigational New Drug
IRB	Institutional Review Board
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NF kB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
NSAIDs	NonSteroidal Anti-Inflammatory Drugs
PI	Principal Investigator
QID	Quater In Die (four times a day)
SAE	Serious Adverse Event

SAP	Statistical Analysis Plan
SOP	Standard Operating Procedure
Th17	T Helper 17 Cells
TID	Ter In Die (three times a day)
QID	Quarter In Die (four times a day)
TLR	Toll-Like Receptor
TIV	Trivalent Inactivated Influenza Vaccine
FNA	Fine Needle Aspiration
CNB	Core Needle Biopsy
PA	Philadelphia
NY	New York
USA	United States of America
CDI	Clostridium Difficile Infection
IgG	Immunoglobulin G
PBMCs	Peripheral Blood Mononuclear Cells
PID	Participant ID
REs	Reactogenicity Events
CTCAE	Common Terminology Criteria for Adverse Events
PCA	Principal Component Analysis
PLSDA	Partial Lease Square Regression
OTUs	Operational Taxonomic Units
SAM	Significance Analysis of Microarrays
FDR	False Discovery Rate

1 BACKGROUND AND RATIONALE

1.1 Background

Vaccination has been one of the most important and cost-effective public health interventions to provide protection against infectious diseases.[1] Since the introduction of the first vaccine in 1796, there have been countless advances in the field. However, numerous gaps remain to be addressed. An important gap is understanding the mechanisms that lead to suboptimal immune responses to vaccination. It has been shown that the magnitude of the immune response produced by vaccines is highly variable among individuals, with both genetic and environmental factors playing an important role. More recently, emphasis is being placed on the role of the microbiota in vaccine immunogenicity.[2] The microbiome is the collection of all microbial cells in and on the human body, with the majority being in the gastrointestinal tract. Studies have shown that germ-free mice, or those treated with antibiotics, have a poorer immune response to infections as compared to mice with unaltered microbiota.[3] It has also been noted that the composition of the microbiome has the ability to affect B and T cell development, which are important aspects of the adaptive immune system and major responders to vaccination.[3]

A study on trivalent inactivated influenza vaccine (TIV) done by Oh et al. (Immunity, 2014) demonstrated the possible role of microbiota in enhancing vaccine immunity, supporting that the microbiome might be a determinant of vaccine efficacy.[4] They show that the immune response to TIV is reduced in mice that lack toll-like receptor 5 (TLR5), which is not a known sensor of viral stimuli, but is usually needed to respond to bacteria's flagellin. This raised the question of the role of bacteria in modulating the immune response against viral vaccines. Therefore, they studied the immune response in germ-free mice and those that were treated with antibiotics, and found that the concentration of TIV-specific IgG and antigen-specific plasma cells in mice exposed to antibiotics was fairly reduced as compared to mice who were not exposed.[4] This further supported the idea that microbiota-derived factors contributed to TLR5 mediated augmentation of immunogenicity of TIV.[4]

Due to this link between microbiome and the immune system, it is important to further understand the impact of the microbiome on the immune response to vaccination. This can be done using systems vaccinology, which is the application of systems biology in vaccinology to predict vaccine

efficacy.[5] The aim is to find molecular signatures, or patterns of gene expression induced after vaccination, which can be used to correlate and predict the development of protective immunity.[6] The goal of our study is to determine whether alteration of microbiota by antibiotic exposure can negatively impact the immunogenicity of rabies vaccine, and to assess the innate and adaptive immune mechanisms responsible for that phenomenon. Imovax® is an inactivated rabies vaccine harvested from infected human diploid cells. The reason behind choosing rabies vaccine in this study is using an antigen that people are naïve to, unlike influenza vaccine which was used in our previous study. To track the response of the immune system to the vaccine, we will draw blood for peripheral blood mononuclear cell (PBMCs) isolation and perform a fine needle aspiration (FNA) or core needle biopsy (CNB) of the draining lymph node, before and after vaccination. Sampling of the draining lymph node has been shown to be a sensitive way to analyze the effector memory T cell repertoire from the skin after administering an intradermal antigen.[7]

In summary, the data from past experiments in mice imply an important role for commensal microbiota in enhancing vaccine-elicited immunity. It is crucial to determine if these effects are also applicable in humans, with the ultimate goal of optimizing vaccine efficacy. Hence, we would like to translate the experimental data on mice to study the impact of antibiotic exposure in humans before vaccination on the elicited immune response to vaccines.

1.2 Rationale and Study Population Selection

The human microbiome plays an important role in the immune response to vaccination and can be altered by the use of antibiotics. Here, we attempt to better understand the immune responses to the inactivated rabies vaccines, with or without use of antibiotics, in subjects who are naïve to rabies vaccination or infection.

Approximately 54 healthy adults with no co-morbidities between the ages of 18-49 years will be recruited from the general population of metro Atlanta and enrolled at the Hope Clinic, Decatur. The above age range was chosen in order to avoid the effect of immune senescence on vaccine efficacy and due to the fact that the rate of *Clostridium difficile* infection is lowest in the age ranged 18-44 years, with less than 2.5 cases per 1,000 discharges.[8]

1.3 Investigational Product(s)/Intervention (s)

The vaccine used in the study will be the Imovax® rabies vaccine manufactured by Sanofi Pasteur (PA, USA).[9] Imovax® is an inactivated rabies vaccine harvested from infected human diploid cells. Subjects in the study will receive the routine adult dose of 1.0 mL given by the intramuscular (IM) route, in the deltoid muscle since vaccination in the gluteal area results in less neutralizing antibodies.[9] Contrary to pre-exposure and post-exposure prophylaxis, where multiple doses of Imovax® rabies vaccine are recommended, only two 1.0 mL dose of Imovax® will be given to participants, since seroprotection is often obtained with only one dose and the subjects are at no risk for acquiring rabies.[9,10] Imovax® will be obtained from the Emory Investigational Drug Service.

The antibiotic regimen will be given 3 days prior to vaccination, on the vaccination day, and one day after vaccination for a total for 5 days. The regimen will include the following antibiotics:

Flagyl® (11) (Pfizer, New York, NY) 500 mg po tid.

Vancocin® (12) (ViroPharma, Exton, PA) 125 mg po qid.

Neomycin sulfate® (13) (X-Gen Pharmaceuticals, Big flats, NY) 500 mg po tid.

All antibiotics will be obtained from the Emory Investigational Drug Service.

1.4 Rationale for Selection of Investigational Product(s)/Intervention(s) and Regimen

The majority of studies demonstrating that antibiotic-mediated depletion of host microbiota influences the development and homeostasis of the immune system employed a specific cocktail of antibiotics consisting of Ampicillin (1 g/L), Neomycin (1 g/L), Vancomycin (0.5 g/L), and Metronidazole (0.5 g/L).

The basis for using this particular combination of antibiotics is to enable a wide spectrum of microbial communities to be targeted for depletion. Indeed, at the indicated doses, this cocktail has been shown to effectively deplete more than 90% of the existing flora in the gut and drastically alter the composition of the microbiota.[14,15]

For this study, we will use a broad spectrum antibiotic regimen affecting all bacteria in the gut flora based on our mice experiments that includes: Vancocin® (with activity against gram positive bacteria), Flagyl® (with activity against anaerobes) and Neomycin sulfate® (with activity against gram negative bacteria).

Additional characteristics of antibiotics selected in the regimen include:

- Poor systemic absorption for Vancocin® and Neomycin sulfate® (3%) when given orally which should result in less systemic side effects.

- Good safety profile for all 3 of these antibiotics used for more than half a century (Flagyl® was developed in 1960, Vancocin® in 1953 and Neomycin sulfate® in 1949).

- Although any antibiotic can cause *Clostridium difficile*-associated colitis, the current regimen includes Vancocin® and Flagyl®, both used at the same cumulative daily dosage to treat *Clostridium difficile* infection but administered in a shorter course of 5 days. For Neomycin sulfate®, the cumulative daily dosages are less than half of the minimum daily dosage used to treat adult hepatic encephalopathy and a 5-day course is same as that required for treating adult hepatic encephalopathy.

This antibiotic regimen (Vancocin®, Flagyl® and Neomycin sulfate®) will be administered three days before vaccination with rabies vaccine, on the day of vaccination and one day post-vaccination in subjects randomized to the antibiotic group. This short course reduces the potential risk of bacterial and fungal superinfection, as well as preventing the development of antibiotic resistance.

1.5 Risks

1.5.1 Risks of Investigational Product(s)/Intervention(s)

Rabies vaccine (Imovax®)

The potential risks of receiving the rabies vaccine include but are not limited to: local reactions such as pain, erythema, soreness, swelling or itching at injection site (25%), systemic reactions such as headache, nausea, abdominal pain, muscle aches and dizziness (20%) and anaphylactic reactions.[9]

Serum sickness type reactions have been reported in persons receiving booster dose (7%); this protocol will provide 2 instead of 3 doses of the vaccine. Also, rare cases of a transient neuroparalytic illness have been reported, which resolved without sequela in 12 weeks, as well a focal subacute central nervous system disorder temporally associated with rabies vaccine. All systemic neuroparalytic or anaphylactic reactions to a rabies vaccine should be immediately reported to VAERS or Sanofi Pasteur Inc.[9]

There may be other unknown side effects.

Antibiotics

Exposure to antibiotics poses a risk for Clostridium difficile infection (CDI), typically resulting in a mild illness with diarrhea, fever and abdominal pain. Severe cases of CDI have resulted in hospital admissions, colectomy, and deaths, mostly in patients with co-morbidities or the elderly. We excluded antibiotics with which CDI has been highly associated with, such as fluoroquinolones.

Flagyl®: The most serious adverse reactions reported in patients treated with Flagyl® have been convulsive seizures, encephalopathy, aseptic meningitis, optic and peripheral neuropathy.[11] Peripheral neuropathy is characterized mainly by numbness or paresthesia of an extremity.[11] The most common adverse reactions reported have been referable to the gastrointestinal tract, particularly nausea, reported by approximately 12% of patients, and can be accompanied by headache, anorexia, and occasionally vomiting; diarrhea; epigastric distress; and abdominal cramping.[11]

Vancocin®: Nephrotoxicity (renal failure, renal impairment, blood creatinine increased) has occurred following oral Vancocin® therapy in randomized controlled clinical studies, and can occur either during or after completion of therapy. The risk of nephrotoxicity is increased in patients >65 years of age.[12]

Ototoxicity has occurred in patients receiving vancomycin.[12] It may be transient or permanent, but has been reported mainly in patients who have been given excessive intravenous doses, who have an underlying hearing loss, or who are receiving concomitant therapy with another ototoxic agent, such as an aminoglycoside.[12]

The most common (>10%) adverse reactions associated with the use of Vancocin® in clinical trials included nausea (17%), abdominal pain (15%) and hypokalemia (13%). Other adverse events (>5%) included peripheral edema, fatigue, fever, diarrhea, vomiting, flatulence, urinary tract infection, back pain and headache. Also, important/life-threatening side effects are rare (<1%) and include vasculitis, thrombocytopenia, nephrotoxicity, neurotoxicity and ototoxicity.[12]

Neomycin sulfate®: Nephrotoxicity, ototoxicity, and neuromuscular blockage have been reported with the use of Neomycin sulfate®.[13] Manifestations of neurotoxicity may include numbness, skin tingling, muscle twitching, and convulsions. The risk of hearing loss continues after drug withdrawal. Subjects concurrently receiving other neurotoxic and/or nephrotoxic drugs may have can have a possible enhancement of the nephrotoxicity and/or ototoxicity of neomycin.[13] Concurrent or serial use of other aminoglycosides and polymyxins may enhance neomycin's nephrotoxicity and/or ototoxicity and potentiate neomycin sulfate's neuromuscular blocking effects. The most common adverse reactions to oral neomycin sulfate are nausea, vomiting and diarrhea.[13]

All three antibiotics in this regimen can lead to the overgrowth of non-susceptible bacteria.

Lidocaine[16]:

Complications of lidocaine (1%) injection used for local anesthesia are rare, mostly seen if a major vessel is inadvertently injected, the recommended dose is exceeded, or as an idiosyncratic response. The immediate side effects are related to the central nervous system (lightheadedness, confusion, tinnitus, blurred or double vision, vomiting, numbness, twitching, tremors, convulsions, unconsciousness, respiratory depression and arrest) and cardiovascular system (decreased myocardial contractility, vasodilation, arrhythmias and cardiac arrest). Allergy to lidocaine either immediate (urticaria or anaphylaxis) or delayed (contact dermatitis) has been described. In adults, the recommended dose is 7 mg/kg with a maximum of 500 mg.

1.5.2 Risk of Study Procedure

The potential risks to subjects are those associated with intramuscular administration of the rabies vaccine, possible reactions to the vaccine, having blood drawn, the risks associated with fine needle aspiration cytology or core needle lymph node biopsy, and exposure to antibiotics.

Blood draws:

Blood sample collection involves transient discomfort and may cause fainting, which is managed by having the subject lie down prior. The blood draw site may bruise, and this can be ameliorated by holding pressure to this site following the blood draw. The sites of blood draw are potential sites of infection, but this risk is made very unlikely by the use of sterile technique.

Fine needle aspiration (FNA) or core needle biopsy (CNB) of the lymph node:

The potential risks for fine needle aspiration and core needle biopsy are rare. They include, but are not limited to: tenderness around aspiration site, bruising, bleeding and hematoma formation, as well as much rarer complications: infection, allergic reaction to anesthetic, vagal reaction, numbness caused by accidental nerve damage, and pneumothorax.[17-19]

1.5.3 Risk of Concomitant Medications, Prophylactic Medications and Rescue Medications

We do not anticipate the use of any other medication; however, should anaphylactic or hypersensitivity reactions occur, epinephrine (1:1000) and diphenhydramine injections are readily available at the Hope Clinic during vaccine use.

Epinephrine injection can be associated with high blood pressure, arrhythmia, lightheadedness, nervousness, restlessness, tremor, shortness of breath and diaphoresis. The frequency of these side effects is not defined. Diphenhydramine injection may be required to treat possible allergic reactions and its use can be associated with low blood pressure, arrhythmia, confusion, dizziness, sedation, restlessness, diarrhea, nausea and urinary retention. The frequency of these side effects is also not defined.

When facing a medical emergency, the clinic staff will follow the institutional SOP by calling 911 first (Hope Clinic) or a code to the hospital on call team (Winship Cancer Institute). If needed, the subject will be transferred to Emory University Emergency Department for further care.

Subjects are allowed to use acetaminophen if they experience a moderate to severe local or systemic side effects after vaccine administration. Subjects are allowed to use acetaminophen if they experience a moderate to severe local or systemic side effects after lymph node sampling.

1.6 Benefits

1.6.1 Benefits of Investigational Product(s)/Intervention(s)

The benefit for the study is predominantly scientific, allowing a better understanding of the role of the microbiome in the immune response to vaccination and the immune responses in the blood and the draining lymph node. However, individuals who receive this vaccine will have some level of protection from potential future rabies exposures.

1.6.2 Benefits of Study Procedure(s)

None.

2 OBJECTIVES

Use of antibiotics can significantly impact the microbiota of patients. The status of the intestinal microbiome may impact the immune responses to vaccinations and alter functional protective immunity in humans. Here we hypothesize that the changes in the fecal microbiome induced by antibiotics translates into suboptimal rabies antibody titers.

2.1 Primary Objective(s)

- Compare antibody titers after vaccination with rabies vaccine in adults with or without use of antibiotics.

2.2 Secondary Objectives

- Evaluate the safety profile of the different antibiotics and the lymph node sampling methods.

2.3 Exploratory Objectives

- Analyze the microbiome in both groups.
- Analyze the repertoire and monoclonal antibodies from plasmablasts in a subset of vaccinees in both groups.
- Identify innate immune signatures (traditional immune parameters measurements + array-based gene expression) and correlate signatures with antibody titers at several times points in both groups.
- Compare the immune profiling in the blood and the lymph node at different time points after vaccination.

3 STUDY DESIGN

This is a single center, mechanistic study with 3 phases in which subjects will be randomized to receive one dose of rabies vaccine at day 4 of a 5 day course of a specific antibiotic regimen (Group A) or rabies vaccine alone (Group B); all subject will receive a second dose of rabies vaccine 28 days later after the first vaccine. The immune responses in the blood and the lymph node will be analyzed.

Phase 1 of the study will determine the best strategy for sampling the lymph node (either by FNA or CNB). The best timing of sampling is determined by phase 2 of the study and if there is a difference in antibody response in the blood in subjects receiving rabies vaccine with or without antibiotics. Phase 3 will compare the difference in immune responses in the blood and lymph node in subjects receiving rabies vaccine with or without antibiotics.

Blood samples for immunologic testing will be collected at screening (from D -35 to D -2), on D0 (at vaccination), D1, D3, D7 (+/- 1 day), D10 (-2/+4), D14 (+/- 2 day), D28 (+/- 5 days), D29, D35 (+/-1 day), D 38(-2; +4) D56 (+/- 5 days), D180 (+/- 14 days), D365 (+/- 14 days) post vaccination for both groups to study innate and/or adaptive immune responses.

Stool samples will be collected in both groups at screening (from D -35 to D -2), on D0 (at vaccination), D1, D3, D7 (+/- 1 day), D10 (-2/+4), D14 (+/- 2 day), D28 (+/- 5 days), D29, D35 (+/- 1 day), D 38(-2; +4); D56 (+/- 5 days), D180 (+/- 14 days), D365 (+/- 14 days) post vaccination to study the gut microbiome.

Antibiotics received by Group A will be started 3 days prior to vaccination (D-3) and continued on day of vaccination (D0) and for one day after vaccination (D1) for a total of 5 days.

- Flagyl® 500 mg po tid
- Vancocin® 125 mg po qid
- Neomycin sulfate® 500mg po tid

The dosage of each antibiotic is taken from their respective package inserts and does not exceed the maximum dose allowed for each antibiotic.

The antibiotic regimen is a broad spectrum regimen covering all types of fecal flora (anaerobes, gram positive, and gram negative bacteria); has a good safety record; has poor systemic absorption for part of the regimen (Vancocin® and Neomycin sulfate®); and part of the regimen is used to treat *Clostridium difficile* infections (Vancocin® and Flagyl®).

Subjects in Group A are asked to avoid all ethanol and any ethanol-containing drugs while taking antibiotics and for 48h before and after taking the antibiotics.

Blood samples for safety laboratory testing (including CBC with differential, creatinine, potassium) will be collected at screening (from D-35 to D -2) and D 7 (+/- 1 day) for both groups (phases 2 and 3).

For Group A, stools will be screened for *Clostridium difficile* carriage by PCR at screening (from D-35 to D-2).

Lymph node sampling will be done once in phase 1. Lymph node sampling will be done 2 times in phase 3 10 days after first and second vaccinations .

3.1 Study Endpoints

3.1.1 Primary Endpoint(s)

- Comparison of antibody titers at D28 post vaccination in both groups.

3.1.2 Secondary Endpoint(s)

- Frequency, severity, and causality of all adverse events in group A subjects and all adverse events related to sampling of the lymph node.

3.1.3 Exploratory Endpoint(s)

- Analyses of microbiome at D0, D1, D3, D7, D14, D28, D35, D56, D180 and D365, and comparison to baseline screening in both groups.

- Analysis of the repertoire and monoclonal antibodies from plasmablasts in a subset of vaccinees at D7 and D35 in both groups.

- Identification of innate immune signatures (traditional immune parameters measurements + array-based gene expression) at D1, D3 and D7 in both groups.

- Correlation of innate immune signatures at days D1, D3 and D7 with antibody titers at D28 and D56 in both groups.

- Description of immune profiling in the blood and the lymph node at different time points.

3.2 Study Completion

This study will be considered “completed” when the primary and secondary objectives have been met.

4 SELECTION OF STUDY SUBJECTS

4.1 Subject Inclusion Criteria (Phase 1)

The target sample size will be 5-8 subjects who will be part of this phases of the study. With the exception of the inclusion/exclusion criteria, there will be no intentional recruitment of particular ethnic or racial groups. Subjects will be screened for eligibility according to the inclusion/exclusion criteria by history. Informed consent will be obtained for study participation.

1. Healthy individuals aged 18-49 years.

2. Able to understand and give informed consent.

4.2 Subject Exclusion Criteria (Phase 1)

1. Receipt of the following:

- Receipt of blood products 3 months prior to procedure.
- Receipt of any vaccine 4 weeks prior to procedure.

2. Presence of co-morbidities or immunosuppressive states such as:

- Chronic medical problems including (but not limited to) insulin dependent diabetes, severe heart disease (including arrhythmias), severe lung disease, severe liver disease, severe kidney disease, auto immune diseases, and grade 4 hypertension*.
- Chronic neurologic conditions including seizure disorder, Parkinson's disease, myasthenia gravis, neuropathy, or history of encephalopathy, meningitis or ototoxicity.
- Alcohol abuse, drug abuse, or psychiatric conditions that in the opinion of the investigator would preclude compliance with the trial.
- Any history of lymphoma involving axillary nodes or any history of breast cancer.
- Impaired immune function or known chronic infections including, but not limited to, known HIV, tuberculosis, hepatitis B or C; organ transplantation (bone marrow, hematopoietic stem cell, or solid organ transplant); immunosuppression due to cancer; current and/or expected receipt of chemotherapy, radiation therapy, steroids** (i.e., more than 20 mg of prednisone given daily or on alternative days for 2 weeks or more in the past 90 days, or high dose inhaled corticosteroids***); and any other immunosuppressive therapies (including anti-TNF therapy), functional or anatomic asplenia, or congenital immunodeficiency.

- Pregnancy or breast feeding.

3. Conditions that could affect the safety of the volunteers, such as:

- History of bleeding disorders or current use of warfarin, aspirin, heparin, nonsteroidal anti-inflammatory drugs (NSAIDs) or other blood thinner/anticoagulant medications in the past week.
- Any allergy to lidocaine.

4. Volunteers with any acute illness, including any fever (≥ 100.4 F [≥ 38.0C], regardless of the route) within 3 days prior to vaccination****.
5. Social, occupational, or any other condition that in the opinion of the investigator might interfere with compliance with the study and vaccine evaluation.
6. Subjects who believe they cannot tolerate the procedure without sedation.
7. Bilateral inflammatory process of upper arms in the past 2 weeks.
8. Prior breast or axillary biopsy and/or surgery.

Note:

*Grade 4 hypertension per CTCAE criteria is defined as life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive).

**Subjects receiving ≥ 20 mg/day of prednisone or its equivalent daily or on alternate days for more than 2 weeks may enter the study after therapy has been discontinued for more than 3 months.

*** Subjects are excluded if on high dose intranasal steroids defined as > 960 mcg/day of beclomethasone dipropionate or equivalent.

****An individual who initially is excluded from study participation based on one or more of the time-limited exclusion criteria (e.g., acute illness, receipt or expected receipt of live or inactivated vaccines) may be reconsidered for enrollment once the condition has resolved as long as the subject continues to meet all other entry criteria.

4.3 Subject Inclusion Criteria (Phases 2&3)

The target sample size will be approximately 46 subjects who will be part of these phases of the study. We will screen 100 subjects to account for screen failures and to reach the enrollment goal. With the exception of the inclusion/exclusion criteria, there will be no intentional recruitment of

particular ethnic or racial groups. Subjects will be screened for eligibility according to the inclusion/exclusion criteria by history. Informed consent will be obtained for study participation.

Subjects eligible to participate shall meet all of the following **inclusion criteria:**

1. Healthy individuals aged 18-49 years.
2. Able to understand and give informed consent.
3. Women of child-bearing potential (not surgically sterile via tubal ligation, bilateral oophorectomy or hysterectomy or who are not postmenopausal for ≥1 year) must agree to practice adequate contraception that may include, but is not limited to, abstinence, monogamous relationship with vasectomized partner, barrier methods such as condoms, diaphragms, spermicides, intrauterine devices, and licensed hormonal methods* for 28 days before and 28 days after Rabies vaccination.

   *Women of child-bearing potential using licensed hormonal methods must also use a second form of contraception.

4.4 Subject Exclusion Criteria (Phases 2&3)

Subjects eligible to participate in phases 2 and 3 shall not meet any of the following **exclusion criteria**:

1. Receipt of the following:

- Receipt of blood products 3 months prior to vaccination or expected receipt through 12 months after vaccination.
- Receipt of any live virus vaccines within 28 days prior to vaccination or expected receipt within 28 days after vaccination.*
- Receipt of any inactivated vaccine within 14 days or expected receipt within 14 days after vaccination other than study vaccine.*
- Receipt of any antibiotic 3 months prior to vaccination or expected receipt 28 days after vaccination.
- Receipt of probiotics and prebiotics 3 months prior to vaccination or expected receipt 28 days after vaccination.
- Receipt of proton pump inhibitors, H2 receptor blockers, or antacids 3 months prior to vaccination or expected receipt 28 days after vaccination.

2. Presence of co-morbidities or immunosuppressive states such as:

- Chronic medical problems including (but not limited to) insulin dependent diabetes, severe heart disease (including arrhythmias), severe lung disease, auto immune diseases, thrombocytopenia and grade 4 hypertension**.
- Chronic neurologic conditions including seizure disorder, Parkinson's disease, myasthenia gravis, neuropathy, or history of encephalopathy, meningitis or ototoxicity.
- Any history of gastrointestinal disease including (but not only): documented bacterial gastroenteritis or gastroenteritis associated with fever or associated with presence of blood/mucus in stools in the last 3 months; inflammatory bowel disease, and/or gastrointestinal surgery.
- Any history of kidney or liver diseases.
- Alcohol abuse, drug abuse, or psychiatric conditions that in the opinion of the investigator would preclude compliance with the trial or interpretation of safety or endpoint data.
- Any history of lymphoma involving axillary nodes or any history of breast cancer.
- Impaired immune function or known chronic infections including, but not limited to, known HIV, tuberculosis, hepatitis B or C; organ transplantation (bone marrow, hematopoietic stem cell, or solid organ transplant); immunosuppression due to cancer; current and/or expected receipt of chemotherapy, radiation therapy, steroids*** (i.e., more than 20 mg of prednisone given daily or on alternative days for 2 weeks or more in the past 90 days , or high dose inhaled corticosteroids****); and any other immunosuppressive therapies (including anti-TNF therapy), functional or anatomic asplenia, or congenital immunodeficiency.
- Pregnancy or breast feeding

3. Conditions that could affect the safety of the volunteers, such as:

- Severe reactions to prior vaccinations, including anaphylaxis
- History of Guillain-Barré syndrome
- History of bleeding disorders or current use of warfarin, aspirin, heparin, nonsteroidal anti-inflammatory drugs (NSAIDs) or other blood thinner/anticoagulant medications in the past week (for subjects undergoing lymph node sampling)
- Use of anticonvulsants
- Use of digoxin or other forms of digitalis
- Any allergy to any component of the vaccine or lidocaine (for subjects undergoing lymph node sampling)

- Allergy to vancomycin, metronidazole or neomycin as well as other aminoglycosides (gentamicin, tobramycin, amikacin, streptomycin)

4. Volunteers with any acute illness, including any fever ($\underline{\geq}$ 100.4 F [$\underline{\geq}$ 38.0C], regardless of the route) within 3 days prior to vaccination *.
5. Social, occupational, or any other condition that in the opinion of the investigator might interfere with compliance with the study and vaccine evaluation.
6. Positive *C difficile* testing by PCR at screening or history of *C difficile* infection.
7. Any grade 2 safety lab test results at screening
8. Previously received any rabies vaccine or immunoglobulin prior to study entry.
9. Are at high risk of exposure to rabies: veterinarians, animal handlers, rabies laboratory workers, spelunkers, frequent contact with rabies virus or with possibly rabid animals, international travelers who are likely to come in contact with animals in parts of the world where rabies is common, and rabies biologics production workers.
10. Bilateral inflammatory process of upper arms in the past 2 weeks.
11. Prior breast or axillary biopsy and/or surgery.

Note:

*An individual who initially is excluded from study participation based on one or more of the time-limited exclusion criteria (e.g., acute illness, receipt or expected receipt of live or inactivated vaccines) may be reconsidered for enrollment once the condition has resolved as long as the subject continues to meet all other entry criteria.

** Grade 4 hypertension per CTCAE criteria is defined as Life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive).

***Subjects receiving $\underline{\geq}$ 20 mg/day of prednisone or its equivalent daily or on alternate days for more than 2 weeks may enter the study after therapy has been discontinued for more than 3 months.

****Subjects are excluded if on high dose intranasal steroids defined as > 960 mcg/day of beclomethasone dipropionate or equivalent.

4.5 Early Study Termination

Subjects may be terminated prior to study completion from the study for the following reasons:

A. The subject elects to withdraw consent from all future study activities, including follow-up.

B. The subject is considered by the PI to be “lost to follow-up” (i.e., no further follow-up is possible because attempts to reestablish contact with the subject have failed).

C. The subject dies.

D. The subject develops a medical condition or is started on new medication(s) not previously mentioned in the list of prohibited medications that, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the subject’s ability to comply with study requirements, or may impact the quality of the data obtained from the study.

E. Blood is not able to be drawn (for technical or other reasons) or the subject does not tolerate multiple blood draw attempts.

F. As deemed necessary by the PI or her designee for noncompliance of any nature.

G. As deemed necessary by the PI after development of a related AE/SAE.

H. The subject becomes pregnant.

Subjects with early termination status are replaced as needed to preserve the statistical power needed to substantiate the primary endpoint (Refer to section 9.1).

Note:

Up to the discretion of the PI, subjects receiving concomitant medications may still continue with scheduled study blood draws. Subjects receiving concomitant medications along with subjects with early termination from this study for any reason may be replaced as needed to preserve the statistical power needed to substantiate the primary endpoint. (Refer to section 9.1)

5 INVESTIGATIONAL PRODUCT(S)/INTERVENTION MATERIAL(S), OTHER STUDY PRODUCTS (CONTROLS/PLACEBOS)

5.1 Investigational Product(s)/Intervention(s)

The Emory Investigational Drug Service will purchase Imovax® (Sanofi Pasteur, PA, USA). The Emory Investigational Drug Service will store the vaccines and will monitor temperatures of the refrigerator(s) containing the vaccines. All antibiotics (Flagyl® (Pfizer, New York, NY); Vancocin® (ViroPharma, Exton, PA); Neomycin sulfate® (X-Gen Pharmaceuticals, Big Flats, NY)) will be purchased as well. Also, lidocaine 1% is used for local anesthesia. Refer to section 1.5, and applicable product labeling, for known and potential risks to human subjects associated with the investigational product(s) intervention(s).

5.1.1 Preparation, administration and dosage

Imovax® Rabies Vaccine

Imovax® rabies vaccine is produced by Sanofi Pasteur SA. It is prepared from the PM-1503-3M strain, and is a stable, sterile, freeze-dried suspension of the rabies virus. The virus is harvested from infected human diploid cells and inactivated by beta-propiolactone. The suspension contains less than 150 mcg neomycin sulfate, less than 100 mg human albumin, 20 mcg of phenol red indicator and less than 50 parts per million of residual beta-propiolactone. After reconstitution, the full 1.0 mL vaccine should be administered immediately intramuscularly. If not given promptly, it should be discarded. The potency of one dose is equivalent to or greater than 2.5 international units of rabies antigen.

5.2 Antibiotics administration and dosage

All antibiotics will be self-administered by the subject by mouth from D-3 until D1 (total of 5 days) as stated below:

- Flagyl® 500 mg po tid

- Vancocin® 125 mg po qid
- Neomycin sulfate ® 500mg po tid

5.3 Accountability of Investigational Product(s)/Intervention(s)

Under Title 21 of the Code of Federal Regulations (21CFR §312.62), the investigator will maintain adequate records of the disposition of the investigational product(s)/intervention material(s), including the date and quantity of the drug received, to whom the drug was dispensed (subject-by-subject accounting), and a detailed accounting of any investigational product(s)/intervention material(s) accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A dispensing log will be kept current for each subject. This log will contain the identification of each subject, the name of the vaccine administered to the subject, the lot of vaccine received by the subject and the date and quantity of vaccine dispensed. A similar log is available for antibiotics dispensed for subjects. A pill count is performed on D0, D1 and D3 to record compliance to the antibiotic regimen. All records regarding the disposition of the investigational product(s)/intervention material(s) will be available for inspection by the site monitor and the health authorities.

5.4 Assessment of Compliance with Investigational Product(s)/Intervention Material(s)

The number of used vaccine syringes will be tracked and reconciled by the Emory Investigational Drug Service.

5.5 Modification or Discontinuation of Investigational Product(s)/Intervention Material(s)

5.5.1 Modification of Investigational Product(s)/Intervention(s)

Unless Imovax® or any of the antibiotics and lidocaine are recalled by the manufacturers, there will be no discontinuation of administration of study vaccine, antibiotics or local anesthesia.

5.5.2 Premature Discontinuation of Investigational Product(s)/Intervention(s)

Refer to sections 4.5 for possible causes of early study termination.

6 OTHER MEDICATIONS

6.1 Concomitant Medications

In accordance with exclusion criteria, subjects expected to receive prohibited medications (see section 6.4) will be considered ineligible for the study. All medications, therapies, or vaccines administered to study subjects after study entry will be documented at each visit.

6.2 Prophylactic Medications

Prophylactic medications will not be administered before vaccination or any other study procedures.

6.3 Rescue Medications

We do not anticipate the use of any rescue medication; however, should anaphylactic or hypersensitivity reactions occur, epinephrine (1:1000) and diphenhydramine injections are readily available at the Hope Clinic during vaccine use and at Winship Cancer Institute during lymph node sampling.

When facing a medical emergency, the clinic staff will follow the institutional SOP by calling 911 first (Hope Clinic) or a code to the hospital on call team (Winship Cancer Institute). If needed, the subject will be transferred to Emory University Emergency Department for further care.

Subjects are allowed to use acetaminophen if they experience a moderate to severe local or systemic side effect after vaccine administration or acetaminophen if they experience a moderate to severe local or systemic side effect after lymph node sampling.

6.4 Concomitant Study Medications

All medications and vaccines received by study subjects after administration of study vaccine should be reported to the study staff and recorded. This includes, but is not limited to, the following:

- blood products, chemotherapy, immunosuppressive therapy (including anti-INF therapy), and radiation therapy (administered at any time after study vaccination).

- inactivated vaccines (administered before the day 14 blood draw).
- live-attenuated vaccine (administered before the day 28 blood draw).
- any antibiotic aside from the study antibiotic (taken before the day 28 blood draw).
- any probiotics and prebiotics (taken before the day 28 blood draw).
- any proton pump inhibitors, H2 receptor blockers, or antacids (taken before the day 28 blood draw).

Any of the above medications could affect the innate, adaptive, or microbiome assay results and should not be used unless medically indicated.

Upon the discretion of the PI, subjects receiving concomitant medications may still continue with scheduled study blood draws. Subjects receiving concomitant medications and early terminated subjects may be replaced as needed to preserve the statistical power needed to substantiate the primary endpoint (refer to section 9.1).

7 STUDY VISITS AND PROCEDURES

7.1 Enrollment and Randomization

This research study will be explained in lay terms to each potential research subject. The potential subject will sign an informed consent form before undergoing any screening study procedures. Subjects who are deemed eligible for the study (see sections 4.1, 4.2, 4.3 and 4.4) will be enrolled and assigned a unique subject number. Enrollment will occur over an approximate 24-month time span. The duration of participation for each subject is approximately 13 months.

Approximately 54 subjects will be included in the study, which occurs in three phases:

Phase 1

Five to eight subjects will undergo both fine needle aspiration (FNA) and core needle biopsy (CNB) of the axillary lymph node during one single visit after screening. No vaccine will be given to these participants. A phone call will be done to ensure subject did not have significant adverse event from the procedure. This will help determine the preferred method of lymph node sampling that will yield the greatest amount of information on the immune response to the vaccine with the lowest rate of adverse events.

Phase 2

Approximately eighteen subjects will be randomized to Group A (receiving antibiotics) or to Group B (not receiving antibiotics) in a 1:1 ratio. All eligible subjects will receive Imovax® at D0 and D28. No FNA was performed as part of Phase 2.

Phase 3

Approximately twenty-two subjects will be randomized to Group A (receiving antibiotics) or to Group B (not receiving antibiotics) in a 1:1 ratio. Approximately 6 subjects in each arm will undergo lymph node sampling at (10 days after the first and second vaccinations).

7.2 Screening Visit(s) (From D-35 until D-2)

Subjects responding to study ads will contact the Hope Clinic for a telephone screening where the eligibility criteria of the subject will be reviewed. If the subject is found to be eligible and continues to be interested in participating after reading the informed consent (emailed or mailed to him/her), a screening appointment will be scheduled. Study staff will review the informed consent form with the subject and will answer all questions related to the study.

Once the subject signs the informed consent, a study participation number will be assigned. The volunteer will be asked to provide demographic information and information related to his/her medical history, including current medication use and vaccination history. The subject's vital signs will be recorded, and a targeted physical exam will be conducted as indicated, based on review of the subject's health status. For female volunteers who are of childbearing potential, a urine pregnancy test will be performed. Only females with a negative urine pregnancy test will be enrolled in the study.

All other study procedures will differ depending on the group (A vs B) and if lymph node sampling is planned or not.

Phase 1: No safety labs.

Phase 2: Eighteen subjects will be randomized into two groups in a 1:1 ratio. Group A will be the group receiving antibiotics, and Group B will be the group not receiving antibiotics. All subjects (Groups A and B) will have blood drawn for safety labs (CBC with differential, creatinine, potassium). All subjects (Groups A and B) will have a stool sample collected from them. Nine subjects (Group A) will be given the three antibiotics and instructed on when and how to start taking the antibiotic pills (D-3 until D1). Subjects in Group A are asked to call the Hope Clinic for any symptom while on antibiotics.

Phase 3: Twenty-two subjects will be randomized into two groups in a 1:1 ratio. All subjects (Groups A and B) will have blood drawn for safety labs (to measure CBC with differential, creatinine, potassium) and approximately 6 will undergo a FNA 10 days after the first and second vaccinations/. All subjects will have a stool sample collected from them. Subjects in Group A will also be given the

three antibiotics and instructed on when and how to start taking the antibiotic pills (D-3 until D1). Subjects in Group A are asked to call the Hope Clinic for any symptom while on antibiotics.

This visit will last approximately 60 minutes to 120 minutes.

7.3 Procedure (screening period or other time points)

Study personnel will first review the subject's current health status, list of medications being taken, and note any change since the screening visit. The subject's vital signs will be recorded. For female volunteers of childbearing potential, a urine pregnancy test will be performed. Only females with a negative urine pregnancy test will get the procedure. Inclusion/exclusion criteria will be also verified before procedure. Subject will have a lymph node sampling (subjects in phase 1 will undergo both CNB and FNA, subjects in phase 3 a FNA, as determined by phase 1).

Phase 1: All subjects will undergo both FNA and CNB at D0 after screening.

Phase 2: No lymph node sampling

Phase 3: Approximately 6subjects from each group (Groups A and B) will undergo lymph node sampling at 10 days after the first and second vaccinations).

Tissue sampling of an axillary lymph node will be carried out percutaneously. The subjects will undergo FNA and/or CNB. Both procedures involve obtaining informed consent, performing a pre-procedure "time out" with the team and subject present to confirm identity subject and the nature of the procedure. Both axillae will be scanned with ultrasound to identify the most accessible node for biopsy. Once this has been identified, the skin will be sterilely prepared, and lidocaine 1% will be injected intra-dermally and subcutaneously to the margin of the node in question, to confer local anesthesia. A small skin incision will be made. Under real-time sonographic guidance, a 21-gauge needle (FNA) or an 18-gauge Bard Marquee automated core biopsy device (core biopsy) (Bard Biopsy, Tempe, AZ) will be placed. For FNA, 2-4 passes will be made to retrieve cytologic material. For core biopsy 3-4 passes will be made to retrieve histologic core samples. After tissue is retrieved, manual pressure will be applied to the biopsy site for 5-10 minutes. The technologist will review post-procedure instructions with the patient. The incision site will be sterilely dressed and the patient will be released after vital signs are checked.

At the end of the visit, the subject will also be instructed to promptly call the site if he/she develops any of the following:

- Illness or treatment from a physician or emergency department; and/or hospitalization due to a complication from the lymph node sampling;
- Development of any adverse event that limits self-care activities of daily living (e.g. bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bed ridden) even if he/she decides not to seek medical care.

Refer to section 8.2 for safety data that must be recorded and reported.

Note: Subjects calling the site for any of the above will receive further instructions on the proper course of action, including a return to the clinic for immediate evaluation, if appropriate.

This visit will last approximately 120 minutes.

7.4 Vaccination visit (D0 and D28)

Study personnel will first review the subject's current health status, list of medications being taken, and note any change since the screening visit. The subject's vital signs will be recorded and a targeted physical exam will be conducted, as indicated by a review of the subject's health status. For female volunteers of childbearing potential, a urine pregnancy test will be performed. Only females with a negative urine pregnancy test will receive the vaccine. Inclusion/exclusion criteria will be also verified before vaccination. Subjects receiving antibiotics will be asked if they have had any symptoms since starting antibiotics, and a pill count is performed.

Blood for immunological assays will be drawn at this visit before administration of the vaccine as well as stool collection. The inactivated rabies vaccine (Imovax®) will be administered intramuscularly in the deltoid region of the preferred arm of the participant. To ensure safety, each subject will be observed for a minimum of 15 minutes following vaccination to note the occurrence of any immediate hypersensitivity reactions. After 15 minutes, the injection site will be examined by study personnel and subject will be asked for systemic adverse reactions.

The subject will be provided with a written description of local and systemic vaccine reactions of mild, moderate and severe intensity (Refer to Appendix B and Appendix C).

At the end of the visit, the subject will also be instructed to promptly call the site if he/she develops any of the following:

- Illness or treatment from a physician or emergency department; and/or hospitalization due to any illness throughout the entire duration of the study;

- Development of any adverse event that limits self-care activities of daily living (e.g. bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bed ridden) even if he/she decides not to seek medical care;

- For subjects receiving antibiotics: development of any of the following symptoms while on antibiotics and throughout the duration of the study:
  - diarrhea (≥ 3 unformed stools/24h) for more than 72h with either fever or abdominal pain*
  - tinnitus**
  - vertigo**
  - decrease hearing**
  - numbness/tingling**

- Beginning or discontinuing any medications/therapies during enrollment in the study.

Refer to section 8.2 for safety data that must be recorded and reported.

Note: Subjects calling the site for any of the above will receive further instructions on the proper course of action, including a return to the clinic for immediate evaluation, if appropriate.

This visit will last approximately 60 minutes.

Note:

*If *C difficile* infection is suspected, *C difficile* PCR testing will be performed. If positive, treatment and follow up for *C difficile* infection will be facilitated.

** if tinnitus, vertigo, decrease hearing, numbness/tingling are present, medical referral will be facilitated.

7.5 In-Person Follow-Up Visits

Phase 1: No follow-up visits are required for subjects from phase 1.

Phases 2 and 3: All subjects will return for the study-related blood draws (innate and adaptive assays) and stool sample collection on Days 1, 3, 7(+/-1), 14(+/-2), D29, 35(+/-1), 56(+/-5), 180 (+/-14), and 365 (+/-14) post vaccination. Study personnel will review the subject's current health status, list of medications being taken, and note any change since the previous visit. The subject's vital signs will be recorded and a targeted physical exam will be conducted, as indicated by a review of the subject's health status. Additionally, safety labs are obtained at D7 visit for both Group A and Group B.

On days 1, 3, 7, 14, 29, 35, 56, 180, and 365, study personnel will review current health status, including:

- Evaluation of local REs and systemic REs of grade 2 or higher severity developing after the previous visit (only until Day 7 Visit) or 7 days after procedure.

- Any grade 2 solicited adverse event (AE) (until D56 or up to 7 days after lymph node sampling) or serious adverse event (SAE) (for the duration of the study) which may not have been reported by the subject by calling the site as directed at a prior visit.

- Development of any adverse event that limits self-care activities of daily living (e.g. bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bed ridden) even if he/she decides not to seek medical care.

- Any medications administered after vaccination.

Subjects receiving antibiotics will also be instructed to call the site if he/she:

- Develops any symptoms while taking antibiotics.

- Develops any of the following symptoms for the duration of the study:
  - diarrhea (≥ 3 unformed stools/24h) for more than 72h with either fever or abdominal pain*
  - tinnitus**
  - vertigo**
  - decrease hearing**
  - numbness/tingling**

A focused physical exam will be conducted (if indicated) based on results of the review of the subject's health status as outlined above.

Note:

*If *C difficile* infection is suspected, *C. difficile* PCR testing will be performed. If positive, treatment and follow up for *C. difficile* infection will be facilitated.

** if tinnitus, vertigo, decrease hearing, numbness/tingling are present, medical referral will be facilitated.

7.6 Telephone Follow-Up Visits

Phase 1: A telephone visit will occur 7 days (+/-2 days) after lymph node sampling unless the subject has a predefined in person visit to the clinic.

Phases 2 and 3: A telephone visit will occur for subjects who received antibiotics (group A) at D-3 and D-2 to inquire if subject started taking antibiotics and if he/she is tolerating them. A telephone visit will occur for subjects who received antibiotics (group A) at D90 (+/- 14 days) to ensure they have not developed *C. difficile* infection. For subjects in phase 3 that undergo FNA, will have 2 additional telephone visits 7 (+/-2) days after each FNA procedure unless the subject has a predefined in person visit to the clinic.

7.7 Visit Windows

Study visits should take place within the time limits below:

D0, D1, D3, D7(+/-1), D14(+/-2), D28(+/-5), D29, D35 (+/-1), D56 (+/-5), D180 (+/-14), and D365 (+/-14). In phase 3 the FNA procedure on D10 has a window of -2/+4 days. If the FNA occurs on D14 then the scheduled follow up visit procedures for D14 will be combined with the FNA visit.

7.8 Study Procedures

Refer to sections 7.1, 7.2, 7.3, 7.4 and 7.5.

7.9 Study Arm Assignment Procedures

7.9.1 Blinding and Randomization

In this study, approximately 54 healthy subjects will be enrolled.

During phase 2, approximately 18 subjects will be randomized to be in either Group A or Group B in a 1:1 ratio.

During phase 3, approximately 22 subjects will be randomized to be in either Group A or Group B in a 1:1 ratio. Six in each group will then undergo FNA -10 days after first and second vaccinations .

The randomization will be performed by the Emory Investigational Drug Service.

7.9.2 Securing Randomization Information

The information on randomization is kept at the Emory Investigational Drug Service.

7.9.3 Requirements for Unblinding

N/A.

7.9.4 Documentation of Unblinding

N/A.

8 SAFETY PROCEDURES

8.1 Stopping Rules

8.1.1 Study Stopping Rules

Study vaccination will be suspended pending expedited review of all pertinent data by the institutional review board and the ISM, if Imovax® or antibiotics (Vancocin®, Flagyl®, and Neomycin sulfate®) or lidocaine were recalled by the manufacturer.

Also, vaccination will be suspended pending review of all pertinent data by the PI and the ISM after the occurrence of any of the following:

1 SAE

1 AE of Grade 4 severity.

2 AEs of Grade 3 severity of similar type other than expected events.

2 grade 2 AEs of abnormal laboratory values at D7.

2 cases of *C difficile* infections in Group A subjects.

1 case of ototoxicity.

1 case of neuropathy.

1 case of neuroparalytic illness.

1 case of infection at the site of lymph node sampling.

2 cases of hematomas at the site of lymph node sampling.

1 case of pneumothorax from lymph node sampling.

1 case of persistent (>4 weeks) paresthesia or numbness in upper extremity due to lymph node sampling.

1 case of persistent (>4 weeks) decreased range of motion due to lymph node sampling.

8.1.2 Individual Subject Stopping Rules

Early study termination will occur in subjects due to any of the following circumstances detailed in section 4.5.

8.1.3 Early Termination From Study Vaccines/ Procedures with Continued Study Participation/Follow-up

Refer to section 4.5.

8.1.4 Follow-up After Early Study Termination

Subjects receiving antibiotics who are prematurely terminated from the study for reasons other than safety events will still be followed to monitor safety for 90 days after the last administered dose of antibiotics.

Subjects who are prematurely terminated from the study due to an AE will be followed until resolution of the AE or until 28 days after a subject terminates from the study, or 7 days after a subject undergoes lymph node sampling in phase 1, whichever comes later. Resolution of an AE is defined as the return to baseline status or as stabilization of the condition with the expectation that it will remain chronic.

After assessing terminated subjects for safety under the provisions stated above, the subject will be seen in clinic, if necessary.

8.1.5 SUBJECT REPLACEMENT

In the case of premature termination (before D28 samples are obtained), extra patients will be recruited, at the discretion of the principle investigator, to maintain the target sample size and the statistical power to substantiate primary endpoint. Please refer to section 9 - Statistical Analysis.

8.2 Adverse Events

This section defines the types of adverse events that may occur, and outlines the procedures for appropriately adverse event collecting, grading, recording, and reporting.

Information in this section complies with 21CFR 312; ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting and ICH E6: Guideline for Good Clinical Practice; and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events Version 4.0 [Published: May 28, 2009; revised version 4.03; June 14. 2010, http://evs.nci.nih.gov/ftp1/CTCAE/About.html]

These criteria have been reviewed by the study investigators and have been determined to be appropriate for this study population.

8.2.1 Safety Reporting

8.2.1.1 Adverse Events (AE)

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An adverse event can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

Any grade 2 adverse event until D28 will be recorded and reported.

8.2.1.2 Suspected Adverse Reaction (SAR)

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

SARs after vaccine administration may include:

- Local reactions: hardening of the skin at the injection site, injection site redness, injection site swelling, pain at the site of injection, bruising of the skin at the injection site.
- Systemic reactions: aching muscles, headache, fever, nausea/vomiting, dizziness.

8.2.1.3 SERIOUS ADVERSE EVENT (SAE) OR SERIOUS SUSPECTED ADVERSE REACTION

An adverse event or suspected adverse reaction is considered ‘‘serious’’ if, in the view of the investigator, it results in any of the following outcomes:

- Death.
- A life-threatening adverse event.
- Inpatient hospitalization or prolongation of existing hospitalization.
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Serious adverse events will be reported and recorded for the entire duration of the study.

Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.2.1.4 Life-Threatening Adverse Event or Life-Threatening Suspected Adverse Reaction

An adverse event or suspected adverse reaction is considered ‘‘life-threatening’’ if, in the view of the investigator, its occurrence places the patient or subject at immediate risk of death.

It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

8.2.1.5 Unexpected Adverse Event or Unexpected Suspected Adverse Reaction

An adverse event or suspected adverse reaction is considered ‘‘unexpected’’ if it is not listed in the Summary of Product Characteristics or is not listed at the specificity or severity that has been observed; or, if the Summary of Product Characteristics is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents.

‘‘Unexpected,’’ as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the Summary of Product Characteristics as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

8.2.1.6 Independent Safety Monitoring

The ISM is a physician with relevant expertise in vaccine trials whose primary responsibility will be to provide independent safety monitoring in a timely fashion and to provide recommendations regarding the safe continuation of this study.

The ISM will evaluate safety data generated from study subjects including all SAEs against the known safety profile of the study vaccine to assess for possible changes to the overall risk of the study.

The ISM will communicate with the PI as needed to discuss any safety events of special interest developing during the study and when conducting the review of the monthly reports of cumulative safety data. The study has provisions for a back-up ISM to ensure that independent safety monitoring happens at all times during the study. Additional roles and responsibilities of the ISM are described in section 8.2.4 below.

8.2.2 Collecting and Recording Adverse Events and Pregnancy

Adverse events may be identified during this study through any of these methods:

1. Examination of the subject during study visits.

2. Questioning the subject during study visits.

3. Receiving a safety report from the subject at any time during the study

Note: subjects will be asked to call the site if they develop any of the following:

- Illness or treatment from a physician or emergency department the entire duration of the study;

- Diarrhea (≥ 3 unformed stools/24h) for more than 72h with either fever or abdominal pain

- Any adverse event that limits self-care activities of daily living (e.g. bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bed ridden) even if he/she decides not to seek medical care;

- Local REs and/or systemic REs of grade 2 or higher severity after the previous visit (only until Day 7 Visit or at day 35 or 7 days after lymph node sampling).

4. Receiving a safety report from subject in Group A if they:

- Develop any symptoms while on antibiotics

- Develop any of the following for the duration of the study:

    - diarrhea (≥ 3 unformed stools/24h) for more than 72h with either fever or abdominal pain

- tinnitus
- vertigo
- decreased hearing
- numbness/tingling

A complete recording of safety events in the CRF will include event term, date(s) of onset and resolution/stabilization, assessment of severity, relationship to study vaccine or procedures/intervention(s) such as phlebotomy, expectedness, determination of whether the AE qualifies as serious or non-serious, treatment required, action taken with study participation, and outcome. AEs qualifying as serious also require a narrative of the event. Updates in safety events will be recorded as additional information becomes available.

Information on pregnancies will be collected from the time a subject signs the consent until the subject completes study participation. Cumulatively monthly reports of safety data will capture any pregnancies and pregnancy outcomes at least on a quarterly basis.

If a subject becomes pregnant after study entry, the investigator will discuss with the subject and/or the treating physician the known possible risks to the fetus.

Subjects becoming pregnant after study entry will be withdrawn from the study and followed until the end of the pregnancy. A pregnancy resulting in congenital anomaly/birth defect will be considered a SAE. Any premature termination of the pregnancy will also be reported and assessed as an SAE as needed.

8.2.3 Grading and Attribution of Adverse Events

8.2.3.1 Grading Criteria

Adverse events will be graded according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events Version 4.0 [May 28, 2009; revised version 4.03; June 14. 2010, http://evs.nci.nih.gov/ftp1/CTCAE/About.html]

This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.

All adverse events whether or not listed in the NCI-CTCAE will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual (A semi-colon indicates 'or' within the description of the grade):

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.

Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.

Grade 4 Life-threatening consequences; urgent intervention indicated.

Grade 5 Death related to AE.

*Instrumental Activities of Daily Living (ADL) refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care Activities of Daily Living (ADL) refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Not all Grades are appropriate for all AEs; therefore, some AEs are listed with fewer than five options for Grade selection.

Anaphylaxis is a disorder characterized by an acute inflammatory reaction resulting from the release of histamine and histamine-like substances from mast cells, causing a hypersensitivity immune response. Clinically, it presents with breathing difficulty, dizziness, hypotension, cyanosis and loss of consciousness and may lead to death.

Severity grading of anaphylaxis as per the NCI-CTCAE manual is as follows:

Grade 1= not applicable

Grade 2= not applicable

Grade 3= Symptomatic bronchospasm, with or without urticaria; parenteral intervention indicated; allergy-related edema/angioedema; hypotension

Grade 4= Life-threatening consequences; urgent intervention indicated

Grade 5= Death

Severity grading of AEs of laboratory abnormalities will be assessed as per the NCI-CTCAE manual.

8.2.3.2 Definition of Attribution

The site investigator will initially determine the relationship of an adverse event to the study vaccine or study procedures (blood draw). The investigator's determination of causality will be used for FDA reporting purposes.

The relationship of an AE to study participation will be determined using definitions in the table below:

Code	Descriptor	Definition (guidelines)
UNRELATED CATEGORY		
1	Unrelated	The adverse event is clearly not related to study. The event is completely related to an etiology other than the study product or study intervention (the alternative etiology must be documented in the study subject's medical record)
2	Unlikely	The adverse event is doubtfully related to study and likely to be related to factors other than study product or study intervention.
RELATED CATEGORIES		
3	Possible	The adverse event may be related to study. There is an association between the event and the administration of study product and there is a plausible mechanism for the event to be related to the study product; there may be also an alternative etiology, such as characteristics of the subject's clinical status and/or underlying disease

4	Probable	The adverse event is likely related to study. There is (1) an association between the event and the administration of study product or study intervention, (2) a plausible mechanism for the event to be related to the study product, and (3) the event could not be reasonably explained by known characteristics of the subject's clinical status and or an alternative etiology is not apparent
5	Definite	The adverse event is clearly related to study. There is (1) an association between the event and the administration of the study product or study intervention, (2) a plausible mechanism for the event to be related to the related to the study product, and (3) causes other than the study product have been ruled out and/or the event re-appeared on re-exposure to the study product

8.2.4 Reporting Serious Adverse Events to the Independent Safety Monitor

The Principal Investigator will notify the ISM by email of any SAE within 24 hours of becoming aware of the event. The initial SAE CRF should be signed by the PI or designee and include as much information as possible.

The SAE case report form will be re-submitted and signed by the PI or designee to the ISM with updated relevant medical information as needed until the event is considered closed by the ISM.

8.2.5 Reporting Serious Adverse Events to the FDA

N/A.

8.2.6 Notifying Institutional Review Board

The Principal Investigator will ensure the timely dissemination of SAE information, including SAEs requiring expedited review by the ISM and to the IRB in accordance with IRB regulations and guidelines.

8.3 Protocol Deviations

Deviations occur when the Investigator, site study staff, or subjects fail to adhere to protocol requirements or when there is non-adherence to GCP as delineated in ICH E6 (R1) guidelines.

Upon determination that a protocol deviation has occurred, the study staff will a) notify the Principal Investigator, and b) will complete the Protocol Deviation form. There could be a discussion between the Principal Investigator and the ISM (if needed) to determine the effect of the protocol deviation on the study subject and his/her further study participation, the effect of the protocol deviation on the overall study, and corrective actions.

The Principal Investigator will complete and sign the Protocol Deviation form and submit it to the ISM if needed and to the site IRB, per IRB regulations.

Identification of protocol deviations will be done through the quality manager at the study site. Reviewing and reporting of minor protocol deviations will be discussed at the Hope Clinic monthly meetings. Major protocol deviations will be discussed with the PI and the ISM, as indicated.

9 SAMPLE SIZE CALCULATIONS AND STATISTICAL PLAN

This is a mechanistic study. Subjects with early termination status are replaced as needed to preserve the statistical power needed to substantiate the primary endpoint

9.1 Sample size and power calculations

The comparison of antibody titers will be based in two ways: direct comparison of antibody titers expressed in international units (IU), and seroprotection rates. Phase 2 and phase 3 subjects will be combined in the analysis, resulting in 20 subjects per treatment group.

For the comparison of the mean log2 antibody titers between the two groups, with 20 subjects in each group, at the alpha level of 0.05, we can reject the null hypothesis of equal means with 80% power if the true effect size ((mean0-mean1)/sigma, assuming equal variance) is 0.91 using an unpaired two-sided t-test.

Based on a previous study of Imovax®, we expect the probability of subjects without antibiotics treatment reach seroprotection is close to 1. Our null hypothesis is the two groups have equal proportions of subjects who will reach seroprotection, and the alternative hypothesis is the antibiotic treatment lowers the proportion of subjects reaching seroprotection. The sample size of 20 subjects per treatment group will allow us 80% power to detect a difference in proportions of 0.31 or higher in a one-sided test of two proportions using a significance level of 0.05. Data Analysis

Data from the study will be analyzed using the statistical software R. High-throughput data will be analyzed using appropriate methods, either by stand-alone software or modules in leading statistical softwares, e.g. the Bioconductor in the R framework. For cytokine data, missing values will be dealt with using multiple imputations. For microarray data, nearest neighbor method and local least squares may be used to fill missing values. Unsupervised learning techniques such as PCA, PLSDA, clustering, and factor analysis will be used for the visualization and identification of global patterns. For descriptive endpoints, we will generate summary statistics, and visualize the data using histograms and boxplots when applicable. For the identification of innate signatures, the two treatment groups will be analyzed separately.

9.1.1 Analyses of Primary Endpoint: Comparison of antibody titers at D28 post vaccination in both groups

Comparison of Antibody Titers at D28 and D56 Post-Vaccination in Both Groups

We will take two alternative routes for data analysis. The first is directly comparing antibody titers between the treatment groups; and the second is comparing the proportions of subjects achieving seroprotection from each group.

The first data analysis route is directly comparing the means/medians of the antibody titers between the two groups at D28 and D56. The antibody titers of each group will be summarized and presented using histograms and strip charts for visual comparison. We will use the t-test to compare the mean/median antibody titers between the two groups. The Wilcoxon test may also be used depending on the distribution of the data.

In addition, we will compare the two groups based on the proportion of achieving seroprotection. For seroprotection, we will dichotomize the outcome into D28 and D56 antibody titer $\geqslant$0.5 IU or D28, D56 antibody titer <0.5 IU. We will then compare the two treatment groups by conducting a Fisher's exact test. We will also report the estimated proportion of seroprotection and its 95% confidence interval for each group.

9.1.2 Analyses of Secondary Endpoint: Frequency, severity, and causality of all adverse events in Group A subjects and all adverse events related to sampling of the lymph node.

Descriptive analysis, e.g. tables and histograms, will be conducted to present severity and causality of all AEs. The frequency will be tabulated. Comparison between the two treatment groups will be made using Fisher's exact test on contingency tables.

Ad hoc analyses of correlations of reactogenicity events with innate and adaptive immune responses may be conducted.

9.1.3 Analysis of Exploratory Endpoints

Exploratory Endpoint 1: Analyses of microbiome at D0, D1, D3, D7, D14, D28, D35, D56, D180, and D365 and comparison to baseline screening in both groups

Sequence results will be analyzed using the open source software package called Quantitative Insights into Microbial Ecology (QIIME).[20] Primary objective of our analyses will be to characterize the composition of microbiota samples and to identify specific microbial communities significantly associated with an experimental group.

Prior to analysis, sequences will undergo quality filtering using QIIME and the sequence quality scores annotated to each raw sequence data. The 16s gene sequences will be aligned and clustered using UCLUST, which is based on a pair-wise identity threshold of 97%.[21] Clustered sequences will be assigned to operational taxonomic units (OTUs).[22] A single representative sequence for each OTU will be aligned using PyNAST to enable taxonomic classification and generation of phylogenetic trees for subsequent analyses.[20,23,24]

OTUs with taxonomic assignments will allow QIIME to assemble a matrix of OTU abundance in each sample. This matrix will then be used to summarize communities by taxonomic composition and abundance, which can be visualized together as area/bar graphs or heatmaps. Information from phylogenetic trees will be used to compute distances or levels of dissimilarity between microbial communities using Unifrac.[25] This tool will enable compositional comparisons between samples (beta diversity) to be conducted. For this purpose, we will use the OTU matrix to calculate distance or dissimilarity of samples between experimental groups using weighted and unweighted UniFrac metrics. QIIME will then generate 2 and 3-dimensional Principal Coordinate Analysis plots to represent the distance matrix data. Furthermore, QIIME codes a tool based on G test of Independence to identify OTUs that are differentially represented across experimental groups. This tool will also be conducted and applied to the beta diversity Principal Coordinate Analyses to help explain which OTUs are significantly associated with a particular experimental group.

For longitudinal analyses of samples within each group, semivariogram plots will be generated using dissimilarity metrics over time (Euclidean) plotted against community dissimilarity (UniFrac).[20,26]

Exploratory endpoint 2: Analysis of the repertoire and monoclonal antibodies from plasmablasts in a subset of vaccinees at D7 and D35 in both groups

The repertoire and monoclonal antibody levels will be presented as descriptions of the actual values. Summary statistics, boxplots and histograms will be used to summarize the data.

Exploratory endpoint 3: Identification of innate immune signatures (traditional immune parameters measurements + array-based gene expression) at D1, D3 and D7 in both groups

We will take the measurements on the day of vaccination as baseline. For traditional immune parameters, we will test if an immune parameter shows significant change over baseline at day 1, 3, and 7 after each vaccination, using a two-sided paired t-test. Fold-change (ratio between the mean values of the two groups) will be combined with the test p-value in a selection criterion as appropriate. Based on previous studies similar in nature, the tentative selection criterion is p-value $\leqslant$ 0.01 and fold change $\geqslant$ 3.

For the gene expression data, we will use the method Significance Analysis of Microarrays (SAM) with paired design to find differentially expressed genes.[27] False discovery rate (FDR) will be used as selection criterion. The tentative selection criterion is FDR $\leqslant$ 0.1.

Exploratory endpoint 4: Correlation of innate immune signatures at days D1, D3 and D7 with antibody titers at D28 and D56 in both groups

For traditional immune parameters, we will calculate the correlation coefficient between the immune parameter and the adaptive immune response (antibody titers at D28 and D56). The p-values associated with the correlation coefficients will be used to select traditional immune parameters that are associated with the respective adaptive immune responses. The tentative selection criterion is p-value $\leqslant$ 0.01.

For gene expression data, we will use the method Significance Analysis of Microarrays (SAM) with quantitative outcome to identify genes that are significantly associated with the respective adaptive immune responses (antibody titers at D28 and D56). False discovery rate (FDR) will be used as selection criterion. The tentative selection criterion is FDR $\leqslant$ 0.1.

Exploratory endpoint 5: Description of immune profiling in the blood and the lymph node at different time points

Data will be presented as descriptions of the actual values. Summary statistics, boxplots and histograms will be used to summarize the data.

9.1.4 Patient Populations

We will use all subjects who are randomized, receive the vaccine, complete at least one day of antibiotic treatment, and have measurements at days 0 and 28 in the data analysis.

9.1.5 Study Subject Baseline Characteristics and Demographics

A summary of descriptive statistics for baseline and demographic characteristics will be provided for all enrolled subjects. Demographic data will include age, race, sex, and medical history, including current medication use and vaccination history.

9.2 Interim Analyses

Interim analysis will be conducted after each phase.

9.3 Deviations from Statistical Plan

The principal features of the study design and of the plan for statistical analysis of the data are outlined in this protocol. Any changes in these principal features will require a protocol amendment and will be described in the final report.

Although our statistical methods and time-points for measurements will adhere to what is proposed herein, for the explorative endpoints, our experience with analyses of similar studies in the past has underscored the need for flexibility and adaptability in trying different statistical approaches to arrive at the most informative results, especially for the high-throughput data.

As such, we may use alternative approaches such as the gene set enrichment analyses, or other approaches, and run additional statistical analyses of data generated by use of assays at time points other than those stated in the primary, secondary and exploratory endpoints, on an ad hoc basis.[28]

10 IDENTIFICATION AND ACCESS TO SOURCE DATA

10.1 Identifying Source Data

The investigator will keep accurate records to ensure that the conduct of the study is fully documented. Data forms are either considered source or protocol-specific CRFs.

10.2 Updating Source Documentation

Documents describing the safety profile of investigational products, such as the investigator's brochure and the package insert, will be amended as needed by the investigational products manufacturer to ensure that the description of safety information adequately reflects any new clinical findings.

The Principal Investigator (or designee) will provide the IRB with the most up-to-date versions of the above documents as soon as the Principal Investigator (or designee) becomes aware of any changes. For purchased investigational products, the Principal Investigator (or designee) will confirm that there are no changes to the package insert every 6 months. In case of package insert changes, the Principal Investigator (or designee) will notify the IRB.

10.3 Permitting Access to Source Data

The investigational site participating in this study will maintain the highest degree of confidentiality for the clinical and research information obtained from the subjects in this study. Medical and research records will be maintained at the study site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site will permit authorized representatives of regulatory authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other subject data may be copied (and all personally identifying information will be removed). Authorized representatives described above are bound to maintain the strict confidentiality of medical and research information that is linked to identify individuals.

11 QUALITY CONTROL AND QUALITY ASSURANCE

The Principal Investigator (or designee) will keep accurate records to ensure that the conduct of the study is fully documented. The investigator will ensure that all CRFs and subject study files are legible and complete for every subject.

When the CRFs are complete, they will be reviewed and signed by the Principal Investigator or designee. All discrepancies identified will be reviewed, and any resulting queries will be resolved with the Principal Investigator (or designee) and the CRFs will be amended as needed.

The Principal Investigator (or designee), through the use of an internal Quality Management Plan, appropriate site quality control, and quality assurance monitoring staff, will be responsible for the regular review of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data and accuracy of source documentation verification. The reports of the internal site monitor will be submitted to the Principal Investigator (or designee).

As per the clinical monitoring plan, site monitoring will be conducted by the independent site monitor in accordance with established Good Clinical Practices (ICH GCP 5.1.1, 5.2, 5.18.1) and the Code of Federal Regulations, as applicable. The overall objectives of site monitoring visits are to ensure:

1. Site compliance with the current version of the approved protocol, consent, documents and local Institutional Review Board requirements.

2. Accuracy and completeness of data entry.

3. Required regulatory documents are current and maintained in the protocol-specific regulatory binder.

4. A designated percent of signed consent forms, inclusion/exclusion criteria, primary, secondary, and tertiary endpoints, safety monitoring parameters, deviations, and serious adverse events are documented.

5. Procedures are in place to administer and monitor study drug / product accountability and documentation of destruction policies.

6. The research staff is adequately trained with respect to GCP and GLP.

7. All observed anomalies or protocol deviations are reported and identify an action plan to minimize study dropouts and non-compliance with defined study procedures.

The results of the site monitoring report will be discussed with the PI, the staff to ensure compliance with the monitor's findings.

12 ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 Statement of Compliance

This study was designed to ensure the protection of subjects according to the ethical principles of the Declaration of Helsinki and amendments concerning medical research in human subjects. This clinical study will be conducted using current good clinical practice (cGCP), as delineated in Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance [1], and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by IRB, as well as any other appropriate health authorities. Any amendments to the protocol or to the consent materials will also be approved by the appropriate bodies listed above prior to implementation.

12.2 Informed Consent Process

The informed consent form will provide information about the study to a prospective subject or subject's legal representative to allow for an informed decision about participation in the study. Prospective subject or subject's legal representative must be given ample opportunity to review the informed consent and inquire about the results of the study. All subjects (or their legally acceptable representative) must read, sign, and date a consent form prior to study participation. Consent materials for subjects who do not speak or read English will be translated into the subjects' appropriate language.

The informed consent form will be revised and receive IRB approval whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent form will be given to a prospective subject for review prior to any study procedure. The Principal Investigator or an approved designee will discuss the consent with the prospective subject and answer questions. The prospective subject will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason.

13 PUBLICATIONS

Publication of any data from this study must be carried out in agreement with the sponsor (Dr Bali Pulendran).

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APPENDICES

APPENDIX A: SCHEDULE OF EVENTS

PHASE 1: (N=5-8)

Day	D-35 D-2	D0	D7 (+/-2 days)
Visit	1	2	3
Informed consent & HIPAA	X		
Demographic and Medical History (including medication and vaccine history).	X		
Verify eligibility	X	X	
PID assignment	X		
Vital signs	X	X	
Focused physical exam (only if indicated based on review of health status)	X	X	
Urine pregnancy test (for female volunteers of childbearing potential)	X	X	
Instruction given to study subjects on safety events		X	
Blood draw for baseline immunology assays		X	
Fine Needle Aspirate of Lymph Node		X	
Core Needle Biopsy of Lymph Node		X	
Telephone call			X

PHASE 2: (N=18, RANDOMIZED 1:1)

Day	D-35 D-2	D -3 D -2	D - 1	D 0	D1	D3	D 7 +/ -1	D1 4 +/- 2	D2 8 +/- 5	D2 9	D3 5 +/- 1	D5 6 +/- 5	D9 0 +/- 14	D18 0 +/- 14	D36 5 +/- 14
Visit	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16
Informed consent & HIPPA	X														
Demographic and Medical History (including medication and vaccine history).	X														
Verify eligibility	X			X					X						
PID assignment	X														
Vital signs	X			X	X	X	X	X	X	X	X	X		X	X
Focused physical exam (only if indicated based on review of health status)	X			X	X	X	X	X	X	X	X	X		X	X
Urine pregnancy test (for female volunteers of childbearing potential)	X			X					X						
Stool sample collection	X	(X)		X	X	X	X	X	X	X	X	X		X	X

Day	D-35 D-2	D -3 D -2	D - 1	D 0	D1	D3	D 7 +/ -1	D1 4 +/- 2	D2 8 +/- 5	D2 9	D3 5 +/- 1	D5 6 +/- 5	D9 0 +/- 14	D18 0 +/- 14	D36 5 +/- 14
Visit	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16
Randomization	X														
Administer antibiotics (Group A)		X	X	X	X										
Assessment of health status while on antibiotics (Group A only)*	X	X		X	X										
Distribute assigned antibiotics and instruct subject when and how to take (Group A only)	X														
Vaccination **				X					X						
Study drug accountability (Group A only)				X	X	X									
Telephone call (Group A only)		X											X		
*** Instruction given to study subjects on	X	X		X	X	X	X	X	X	X	X	X		X	X

Day	D-35 D-2	D -3 D -2	D - 1	D 0	D1	D3	D 7 +/ -1	D1 4 +/- 2	D2 8 +/- 5	D2 9	D3 5 +/- 1	D5 6 +/- 5	D9 0 +/- 14	D18 0 +/- 14	D36 5 +/- 14
Visit	1	2	3	4	5	6	7	8	9	10	12	13	14	15	16
safety events and concomitant medications.															
Assessment of health status after administration of study vaccine					X	X	X	X	X	X	X	X		X	X
Blood draw for Innate Assays and Adaptive Assays ****				X	X	X	X	X	X	X	X	X		X	X
Blood draw for safety labs*****	X						X								

Footnotes:
*Subjects in Group A are asked to report any symptoms experienced while on antibiotics (from D-3 until D1).
**Any Adverse Event (including – but not limited to- vaccine reactions and local or systemic reactogenicity Events) of grade 2 or higher severity or serious adverse event (SAE) occurring after vaccination while the subject is still at the clinical site will be recorded and reported.
*** Instruction includes the following:

1. Subjects will be provided (on Day 0 only) with a written description of local and systemic vaccine reactions of mild, moderate and severe intensity and instructed to call the site to report reactogencity events of grade 2 or higher severity within 1 week following vaccination or procedure.
2. Subjects will also be instructed to promptly call the site if he/she develops any of the following:
   - Illness or treatment from a physician or emergency department; and/or hospitalization due to any illness throughout the entire duration of the study;
   - Any adverse event that limits self-care activities of daily living (e.g. bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bed ridden) even if he/she decides not to seek medical care; or
   - Any of the following symptoms for the duration of the study (Group A subject only):
     - diarrhea (≥ 3 unformed stools/24h) for more than 72h with either fever or abdominal pain
     - tinnitus
     - vertigo
     - decrease hearing
     - numbness/tingling
   - he/she starts/stops medications during enrollment in the study.

**** Innate assays will not be conducted at day 28 and at later timepoints. Adaptive assays will not be conducted at days 1 and 3.
*****Safety labs include CBC with differential, creatinine, and potassium.

PHASE 3: (N=22, RANDOMIZED 1:1)

Day	D-35 D-2	D-3 D-2	D-1	D0	D1	D3	D7 +/- 1	D10 -2/+4	D17+/-2	D28 +/- 5	D35 +/- 1	D38 -2/+4	D45+/-2	D56 +/- 5	D90	D180 +/- 14	D365 +/- 14
Visit	1	2	3	4	5	6	7	8		9	10	11		12	13	14	15
Informed consent & HIPPA	X																
Demographic and Medical History (including medication and vaccine history).	X																
Verify eligibility	X			X						X							
PID assignment	X																
Vital signs	X			X	X	X	X	X		X	X	X		X		X	X
Focused physical exam (only if indicated based on review of health status)	X			X	X	X	X	X		X	X	X		X		X	X
Urine pregnancy test (for female volunteers of	X			X						X							

Day	D-35 D-2	D-3 D-2	D-1	D0	D1	D3	D7 +/- 1	D10 -2/+4	D17+/-2	D28 +/- 5	D35 +/- 1	D38 -2/+4	D45+/-2	D56 +/- 5	D90	D180 +/- 14	D365 +/- 14
Visit	1	2	3	4	5	6	7	8		9	10	11		12	13	14	15
childbearing potential)																	
Stool sample collection	X	(X)		X	X	X	X	X		X	X	X		X		X	X
Randomization	X																
Administer antibiotics (Group A)		X	X	X	X												
Assessment of health status while on antibiotics (Group A only)*	X	X		X	X												
Distribute assigned antibiotics and instruct subject when and how to take (Group A only)	X																
Vaccination **				X						X							
Study drug accountability (Group A only)				X	X	X											
Telephone call (Group A only)									X				X		X		

Day	D-35 D-2	D-3 D-2	D-1	D0	D1	D3	D7 +/- 1	D10 -2/+4	D17+/-2	D28 +/- 5	D35 +/- 1	D38 -2/+4	D45+/-2	D56 +/- 5	D90	D180 +/- 14	D365 +/- 14
Visit	1	2	3	4	5	6	7	8		9	10	11		12	13	14	15
*** Instruction given to study subjects on safety events and concomitant medications.	X	X		X	X	X	X	X		X	X	X		X		X	X
Assessment of health status after administration of study vaccine					X	X	X	X		X	X	X		X		X	X
Blood draw for Innate Assays and Adaptive Assays ****				X	X	X	X	X		X	X	X		X		X	X
Blood draw for safety labs*****	X						X										
Fine Needle Aspirate or Core Needle Biopsy******								X				X					

Footnotes:

*Subjects in Group A are asked to report any symptoms experienced while on antibiotics (from D-3 until D1).

**Any Adverse Event (including – but not limited to- vaccine reactions and local or systemic Reactogenicity Events) of grade 2 or higher severity or serious adverse event (SAE) occurring after vaccination while the subject is still at the clinical site will be recorded and reported.

*** Instruction includes the following:

1. Subjects will be provided (on Day 0 only) with a written description of local and systemic vaccine reactions of mild, moderate and severe intensity and instructed to call the site to report reactogencity events of grade 2 or higher severity within 1 week following vaccination.

2. Subjects will also be instructed to promptly call the site if he/she develops any of the following:
    - Illness or treatment from a physician or emergency department; and/or hospitalization due to any illness throughout the entire duration of the study;
    - Any adverse event that limits self-care activities of daily living (e.g. bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bed ridden) even if he/she decides not to seek medical care; or
    - Any of the following symptoms for the duration of the study (Group A subject only):
        - diarrhea (≥ 3 unformed stools/24h) for more than 72h with either fever or abdominal pain
        - tinnitus
        - vertigo
        - decrease hearing
        - numbness/tingling
    - He/she starts/stops medications during enrollment in the study.

**** Innate assays will not be conducted at day 28 and at later timepoints. Adaptive assays will not be conducted at days 1 and 3.

***** Safety labs include CBC with differential, creatinine, and potassium.

******FNA will be performed 10 days after each vaccination; D10 and D14 can be combined. A telephone visit will occur 7 days (+/-2 days) after lymph node sampling unless the subject has a predefined in person visit to the clinic.

APPENDIX B. SEVERITY SCALE FOR LOCAL VACCINE REACTIONS (LOCAL REACTOGENICITY EVENTS)

	INJECTION SITE REACTIONS		
	Grade		
	1	**2**	**3**
Swelling/ Induration/	Mild induration, able to move skin parallel to plane (sliding) and perpendicular to skin (pinching up)	Moderate induration, able to slide skin, unable to pinch skin; limiting instrumental activities of daily living	Severe induration, unable to slide or pinch skin; limiting arm movement limiting self-care activities of daily living
Redness/ erythema	Asymptomatic or mild symptoms; intervention not indicated	Moderate; minimal, local; limiting age-appropriate instrumental activities of daily living	Severe but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self-care activities of daily living
Pain/tenderness	Mild	Moderate pain; limiting instrumental activities of daily living	Severe pain; limiting self-care activities of daily living
Pruritis (Itching)	Mild	Moderate itching limiting daily activity	Severe itching limiting daily activity

Note:
Instrumental Activities of Daily Living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
Self-care Activities of Daily Living refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

APPENDIX C. SEVERITY SCALE FOR GENERALIZED VACCINE REACTIONS (SYSTEMIC REACTOGENICITY EVENTS)

	GENERAL ADVERSE REACTIONS		
	Grade		
	1	2	3
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity
Muscle aches (myalgia)	No interference with activity	Some interference with activity	Significant; prevents daily activity
Dizziness	No interference with activity	Some interference with activity	Significant; prevents daily activity
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
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
Abdominal pain	No interference with activity	Some interference with activity	Significant; prevents daily activity
Fever	38.0 - 39.0 degrees C (100.4 - 102.2 degrees F)	>39.0 - 40.0 degrees C (102.3 - 104.0 degrees F)	>40.0 degrees C (>104.0 degrees F) for <=24 hrs

Note:
Instrumental Activities of Daily Living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
Self-care Activities of Daily Living refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden

APPENDIX D: SITE CLINICAL QUALITY MANAGEMENT PLAN

EMORY UNIVERSITY CLINICAL QUALITY MANAGEMENT PLAN
VERSION 8.0, 11-DEC 2020

1.0 SITE IDENTIFICATION

1.1 SITE IDENTIFICATION:

THE HOPE CLINIC OF THE EMORY VACCINE CENTER, 500 IRVIN COURT, SUITE 200, DECATUR, GA 30030

THE HOPE CLINIC OF THE EMORY VACCINE CENTER, 484 IRVIN COURT, SUITE 230, DECATUR, GA 30030

EMORY CHILDREN'S CENTER VACCINE RESEARCH CLINIC (ECC-VRC), 2015 UPPERGATE DR, ATLANTA, GA 30322

EMORY UNIVERSITY HOSPITAL, 1364 CLIFTON RD NE, ATLANTA, GA 30322

EMORY UNIVERSITY HOSPITAL-MIDTOWN, 550 PEACHTREE ST NE, ATLANTA, GA 30308 EMORY SAINT JOSEPH HOSPITAL, 5665 PEACHTREE DUNWOODY RD, ATLANTA, GA 30342 EMORY DECATUR HOSPITAL, 2701 N DECATUR RD, DECATUR, GA 30033
GRADY MEMORIAL HOSPITAL, 80 JESSE HILL JR. DRIVE SE, ATLANTA, GA 30303

THE ATLANTA VETERANS AFFAIRS (VA) MEDICAL CENTER, 1670 CLAIRMONT ROAD, DECATUR, GA 30033

1.2 WHEN VTEU/IDCRC STUDIES ARE CONDUCTED AT THE SITES LISTED ABOVE OUR CQMP PLAN WILL BE FOLLOWED.

2.0 SCOPE AND RESPONSIBILITY

THE QUALITY MANAGERS AT THE HOPE CLINIC AND EMORY CHILDREN'S CENTER VACCINE RESEARCH CLINIC (ECC-VRC) AT EMORY UNIVERSITY HAVE BEEN DESIGNATED BY THE PRINCIPAL INVESTIGATOR, NADINE ROUPHAEL, MD AND EVAN ANDERSON, MD TO DEVELOP, IMPLEMENT, AND OVERSEE ALL FUNCTIONS OF THIS QUALITY MANAGEMENT PLAN FOR FEDERALLY FUNDED AND HUMAN SUBJECTS RESEARCH/CLINICAL TRIALS. PER INTERNATIONAL COUNCIL FOR HARMONIZATION (ICH) GCP E6 (R2) THE INVESTIGATOR IS RESPONSIBLE FOR SUPERVISING ANY INDIVIDUAL OR PARTY TO WHOM THE INVESTIGATOR DELEGATES STUDY TASKS CONDUCTED AT THE TRIAL SITE.

NADINE ROUPHAEL, MD IS RESPONSIBLE FOR THE QUALITY MANAGEMENT PLAN AT THE HOPE CLINIC AND EVAN ANDERSON, MD, AT THE ECC-VRC.

THE QUALITY MANAGER ENSURES DELEGATED STAFF AND RESPECTIVE FUNCTIONS ARE DOCUMENTED ON THE STUDY PERSONNEL/SITE RESPONSIBILITY-SIGNATURE LOG OR EQUIVALENT DELEGATION OF RESPONSIBILITY LOG VIA

QUALITY ASSURANCE (QA) REVIEW OF EACH INSTITUTIONAL REVIEW BOARD (IRB) APPROVED STUDY PRIOR TO INITIATION OF ENROLLMENT AND IMMEDIATELY PRIOR TO STUDY TERMINATION.

3.0 CLINICAL QUALITY MANAGEMENT PROCESS DESCRIPTION

3.1 PER ICH E6 (R2), INVESTIGATOR SECTION 4.9 RECORDS AND REPORTS, THE INVESTIGATOR SHOULD MAINTAIN ADEQUATE AND ACCURATE SOURCE DOCUMENTS (SD) AND TRIAL RECORDS THAT INCLUDE ALL PERTINENT OBSERVATIONS ON EACH OF THE SITE'S TRIAL SUBJECTS. SOURCE DATA SHOULD FOLLOW A.L.C.O.A.C. PRINCIPLES (ATTRIBUTABLE, LEGIBLE, CONTEMPORANEOUS, ORIGINAL, ACCURATE, AND COMPLETE). CHANGES TO SOURCE DATA SHOULD BE TRACEABLE, SHOULD NOT OBSCURE THE ORIGINAL ENTRY AND SHOULD BE EXPLAINED, IF NECESSARY, (E.G, VIA AN AUDIT TRAIL). THESE GUIDELINES/PRINCIPLES WILL BE APPLIED TO ALL FEDERALLY FUNDED ACTIVITIES, AS WELL AS ALL HUMAN SUBJECT RESEARCH STUDIES AND CLINICAL TRIALS PREFORMED AT THE HOPE CLINIC AND THE ECC-VRC. ALL RECORDS WILL BE KEPT IN AUDIT READY PREPAREDNESS WITH EMPHASIS ON WRITTEN PROCESSES (STANDARD OPERATING PROCEDURES (SOPS), SOURCE DOCUMENTS, CORRECTIVE AND PREVENTIVE ACTION PLANS (CAPA) AND HOW THEY WERE CARRIED OUT AND/OR EVALUATED.

3.2 QUALITY CONTROL (QC) ACTIVITIES: QUALITY CONTROL IS DEFINED AS THE REAL TIME, "DAY-TO-DAY", OBSERVATION AND DOCUMENTATION OF THE SITES' WORK PROCESSES TO ENSURE THAT ACCEPTED PROCEDURES ARE FOLLOWED.

3.2.1 APPLICABLE SITE STANDARD OPERATING PROCEDURES WILL BE FOLLOWED SUCH AS:

- INFORMED CONSENT PROCESS
- REPORTING OF ABNORMAL LABORATORY RESULTS
- SOURCE DOCUMENTATION GUIDELINES
- DIRECT DATA ENTRY (DDE) GUIDELINES (SEE DIRECT DATA ENTRY (DDE) SOP)

3.2.2 ROLES/RESPONSIBILITIES: AT EACH CLINIC VISIT THE CLINICAL STAFF RECORD DATA ON APPROPRIATE SOURCE DOCUMENTS. THE QM MANAGER, OR DESIGNEE, IS RESPONSIBLE FOR CONDUCTING QUALITY CONTROL REVIEW OF CASE REPORT FORMS (CRFS) AND SDS FOR COMPLETENESS AND CORRECTNESS. A QM MANAGER, OR DESIGNEE, WILL NOT REVIEW HIS/HER OWN DOCUMENTATION.

3.2.2.1 QUALITY MANAGER, OR DESIGNEE, WILL REVIEW 100% OF INFORMED CONSENT DOCUMENTS (ICFS) AND CASE REPORT FORMS (CRFS) FOR ACCURACY, COMPLETENESS, AND TO ENSURE PROPER DATING AND SIGNING. THE A.L.C.O.A.C. PRINCIPLES FOR APPROPRIATE DOCUMENTATION WILL BE EMPLOYED IN OUR QC ACTIVITIES. CRFS WILL BE SUBMITTED TO THE SPONSOR WITHIN THE TIMEFRAME REQUESTED BY THE SPONSOR, 3 BUSINESS DAYS.

3.2.2.2 ERRORS WILL BE QUANTIFIED AND PRESENTED AS THE NUMBER OF ERRORS/100 SOURCE DOCUMENT PAGES REVIEWED. DIRECT DATA ENTRY ERRORS WILL BE QUANTIFIED BY DATA SYSTEM CHARACTERISTICS AND ESTABLISHED AT STUDY ONSET.

3.2.2.3 IDENTIFIED ERRORS WILL BE BROUGHT TO THE ATTENTION OF THE APPROPRIATE STAFF MEMBER FOR CORRECTION ON THE SOURCE AND/OR ELECTRONIC DATA CAPTURE (EDC) DOCUMENT WITHIN 3 BUSINESS DAYS OF THE ERROR(S) BEING IDENTIFIED.

3.2.2.4 THE QUALITY CONTROL REVIEW TOOL (APPENDIX C) WILL BE COMPLETED BY THE QM MANAGER OR DESIGNEE. THIS LOG IDENTIFIES AND TRACKS CATEGORIES OF ICF AND CRF ERRORS. THIS INFORMATION WILL BE AGGREGATED AND REPORTED TO THE SITE STAFF, USING THE MONTHLY QC ERROR REPORT (APPENDIX D) AT THE MONTHLY QM MEETING.

3.2.2.4.1.1 INFORMATION COLLECTED AND DOCUMENTED ON APPENDIX D INCLUDE:
3.2.2.4.1.1.1 REVIEWER INITIALS (PERFORMED BY QM
MANAGER OR DESIGNEE.
3.2.2.4.1.1.2 REVIEWER DATE.
3.2.2.4.1.1.3 PARTICIPANT IDENTIFICATION NUMBERS (PID) REVIEWED.
3.2.2.4.1.1.4 SPECIFIC INDICATORS REVIEWED. 3.2.2.4.1.1.5 FINDINGS/RESULTS OF REVIEW.
3.2.2.4.1.1.6 DATE ERRORS/FINDINGS WERE CORRECTED.

3.2.2.5 CLINICAL QUERY REQUESTS WILL BE REVIEWED AND ANSWERED BY THE STUDY COORDINATOR, OR A DESIGNEE, OR REFERRED TO THE ATTENTION OF THE APPROPRIATE STAFF MEMBER FOR A RESPONSE. THE STUDY COORDINATOR, OR DESIGNEE, WILL PROVIDE THE RESPONSE TO THE SPONSOR WITHIN THE REQUIRED TIME-FRAME INDICATED BY THE SPONSOR. THE STUDY COORDINATOR, OR DESIGNEE, WILL MAINTAIN COPIES OF QUERY RESPONSES IN THE STUDY FILES.

3.2.2.6 THE ABOVE TOOLS WILL BE USED TO IDENTIFY TRENDS AND/OR ISSUES OF CONCERN. THESE TRENDS WILL BE PRESENTED AND DISCUSSED WITH THE STAFF MEMBERS AT THE MONTHLY QM MEETING OR SOONER IF INDICATED.

3.2.2.7 CORRECTIVE ACTION WILL BE TAKEN AS NEEDED TO ADDRESS AREAS OF CONCERN. SEE SECTION 9.5 CORRECTIVE AND PREVENTIVE ACTIONS.

3.2.2.8 EVALUATION OF THE CAPA WILL TAKE PLACE AS PART OF THE ONGOING QM PROCESS. SEE SECTION 9.5 CORRECTIVE AND PREVENTIVE ACTIONS.

3.2.3 RECORD SELECTION: METHODS FOR RECORD SELECTION FOR REVIEW ARE AS FOLLOWS:

3.2.3.1 NEW PROTOCOLS: RECORDS (E.G., ICFS, CRFS – PAPER AND/OR ELECTRONIC, CLINICAL LABORATORY REPORTS, SPECIMEN LOGS, CLINIC NOTES, VOLUNTEER CHARTS, AND OTHER SOURCE DOCUMENTS) OF THE FIRST FIVE VOLUNTEERS ENROLLED IN EACH PROTOCOL WILL RECEIVE A 100% REVIEW. NEW PROTOCOLS WILL BE GIVEN PRIORITY OVER OPEN PROTOCOLS FOR REVIEW.

3.2.3.2 PROTOCOLS INVOLVING STUDY PRODUCT AND/OR A PROCEDURE: QUALITY MANAGER, OR DESIGNEE, WILL REVIEW, IN REAL-TIME, 100% OF INFORMED CONSENT DOCUMENTS (ICFS) AND CASE REPORT FORMS (CRFS) FOR ACCURACY, COMPLETENESS, AND TO ENSURE PROPER DATING AND SIGNING. THE A.L.C.O.A.C. PRINCIPLES FOR APPROPRIATE DOCUMENTATION WILL BE EMPLOYED IN OUR QC ACTIVITIES.

3.2.3.3 NEW CLINICAL RESEARCH STAFF: 100%, BUT NOT LESS THAN FIVE, OF ALL VISITS COMPLETED BY A NEW MEMBER, WILL RECEIVE A PROMPT, “REAL-TIME”, REVIEW UNTIL COMPETENCY IS DETERMINED.

3.2.3.4 HIGH RISK EMERGENT PROTOCOLS: PROTOCOLS THAT ARE CONSIDERED TO BE HIGH RISK OR CONDUCTED UNDER EMERGENT CONDITIONS WILL FOLLOW THE SAME PROCEDURE OF “REAL-TIME” REVIEW AND WILL BE REVIEWED WITHIN ONE (1 BUSINESS DAY OF THE VISIT. 100% OF ALL ICFS AND CRFS WILL BE REVIEWED,

3.2.3.5 HIGH ACCRUING PROTOCOLS: BASED ON THE RECOMMENDATIONS OF THE PI AND/OR STUDY COORDINATOR, HIGH ENROLLING PROTOCOLS MAY BE TARGETED FOR AN EARLY “REAL-TIME” OR MORE THOROUGH REVIEW. 100% OF ALL ICFS AND CRFS WILL BE REVIEWED.

3.2.3.6 SCREEN FAILURE/CONSENT WITHDRAWAL: ALL SCREEN FAILURES/CONSENT WITHDRAWAL RECORDS WILL BE REVIEWED, IN “REAL-TIME”, TO ASSURE APPROPRIATE INFORMED CONSENT PROCESS AND DOCUMENTATION OF THE REASON FOR SCREEN FAILURE OR CONSENT WITHDRAWAL.

3.2.4 QUALITY CONTROL TOOLS (TOOLS, CHECKLISTS AND REMINDERS) THE TOOLS FOR QC ARE ATTACHED.

3.2.4.1 INTERNAL (SITE-SPECIFIC) SOURCES:

3.2.4.1.1 THE EMORY UNIVERSITY QUALITY CONTROL REVIEW TOOL (APPENDIX C). INFORMATION REGARDING STUDY VISIT SCHEDULE/PROCEDURES, ELIGIBILITY CHECKLIST, AND INFORMED CONSENT ARE REVIEWED USING THIS TOOL.

3.2.4.1.2 THE EMORY UNIVERSITY MONTHLY QC ERROR REPORT (APPENDIX D). INTERNAL QA/QC FINDINGS, SUMMARY REPORTS ARE REVIEWED AND REPORTED USING THIS TOOL.
3.2.4.1.3 THE EMORY UNIVERSITY ANNUAL QUALITY MANAGEMENT REPORT (APPENDIX F)
3.2.4.1.4 THE EMORY VTEU SUMMARY REPORT LOG (APPENDIX G)

3.2.4.2 EXTERNAL SOURCES:
3.2.4.2.1 DATA ENTRY, QUERY/ERROR, OR TRANSMISSION REPORTS FROM THE DATA MANAGEMENT CENTER
3.2.4.2.2 CLINICAL SITE MONITORING REPORTS

3.3 QUALITY ASSURANCE (QA) ACTIVITIES:

QA IS DEFINED AS THE PERIODIC, SYSTEMATIC, OBJECTIVE, AND COMPREHENSIVE EXAMINATION OF THE TOTAL WORK EFFORT TO DETERMINE THE LEVEL OF COMPLIANCE WITH GOOD CLINICAL PRACTICE (GCP) STANDARDS. QA ACTIVITIES SHOULD ALSO BE PERFORMED. THE HOPE CLINIC AND ECC-VRC WILL PARTICIPATE IN QUALITY ASSURANCE ACTIVITIES IDENTIFIED BY THE INFECTIOUS DISEASES CLINICAL RESEARCH CONSORTIUM (IDCRC).

AT EACH CLINIC VISIT THE CLINICAL STAFF RECORD DATA ON APPROPRIATE SOURCE DOCUMENTS. THE QUALITY MANAGER, OR DESIGNEE, IS RESPONSIBLE FOR CONDUCTING QA REVIEWS OF THE SOURCE DOCUMENTS TO ENSURE THE ADHERENCE TO POLICIES AND PROCEDURES, THE STUDY PROTOCOL, AND THE ACCURACY OF RESEARCH RECORDS. A QM MANAGER, OR DESIGNEE, WILL NOT REVIEW HIS/HER OWN DOCUMENTATION.

THE FOLLOWING ONGOING ACTIVITIES WILL BE CONDUCTED AT THE SITE AS PART OF THE QA PROCESS:

3.3.1 ROLES/RESPONSIBILITIES: AT EACH CLINIC VISIT THE CLINICAL STAFF RECORD DATA ON APPROPRIATE SOURCE DOCUMENTS. THE QM MANAGER, OR DESIGNEE, IS RESPONSIBLE FOR CONDUCTING QUALITY ASSURANCE REVIEWS OF THE SOURCE DOCUMENTS FOR THE FIRST FIVE PARTICIPANTS ENROLLED IN A STUDY TO ENSURE THE ADHERENCE TO POLICIES AND PROCEDURES, THE STUDY PROTOCOL, AND THE ACCURACY OF THE RESEARCH RECORDS. THE QM MANAGER, OR DESIGNEE, WILL ALSO PERFORM REVIEW(S), REAL-TIME, PERIODIC, , AND ON A RANDOM BASIS, DURING THE COURSE OF THE STUDY. THE QM MANAGER, OR DESIGNEE, IS RESPONSIBLE FOR CONDUCTING QUALITY ASSURANCE REVIEW OF CRFS AND SDS. A QM MANAGER, OR DESIGNEE, WILL NOT REVIEW HIS/HER OWN DOCUMENTATION.

3.3.2 RECORD SELECTION: SD DATA ENTRIES WILL BE REVIEWED BY THE QM MANAGER, OR DESIGNEE, USING QA REVIEW TOOL (APPENDIX A). THE QA REVIEW TOOL INCLUDES THE KEY INDICATORS FOR QA AS LISTED IN SECTION 4.0.

3.3.2.1 METHODS OF RECORD SELECTION FOR QA REVIEW ARE AS FOLLOWS:

3.3.2.1.1 SAMPLE SIZE (MINIMUM PERCENTAGE) MONTHLY QA REVIEWS WILL CONSIST OF A RANDOM SELECTION OF 10%, AT A MINIMUM, OF CLINIC RECORDS (E.G. CASE REPORT FORMS – PAPER AND/OR ELECTRONIC, CLINICAL LABORATORY REPORTS, SPECIMEN LOGS, CLINIC NOTES, VOLUNTEER CHARTS, AND OTHER SOURCE DOCUMENTS), ALTERNATING EXISTING OPEN PROTOCOLS TO ASSURE REVIEW OF ALL ACTIVE PROTOCOLS OVER THE COURSE OF THE CALENDAR YEAR. THIS PERCENTAGE MAY INCREASE BASED ON FINDINGS AND/OR PROBLEMS WITH PROTOCOL(S).

3.3.2.1.2 HIGH RISK PROTOCOLS – PROTOCOLS THAT ARE CONSIDERED TO BE HIGH RISK WILL BE TARGETED FOR AN EARLY AND MORE THOROUGH REVIEW THAN THE SAMPLE SIZE DESCRIBED ABOVE.

3.3.2.1.3 HIGHER ACCRUING PROTOCOLS – BASED ON THE RECOMMENDATIONS OF THE PI AND/OR STUDY COORDINATOR, HIGH ENROLLING PROTOCOLS MAY BE TARGETED FOR AN EARLY OR MORE THOROUGH REVIEW.

3.3.2.1.4 INITIAL ENROLLMENT IN NEW PROTOCOLS – THE FIRST FIVE (5) ENROLLED PARTICIPANT RECORDS FOR EACH NEW PROTOCOL WILL RECEIVE A 100% QA AUDIT.

3.3.2.1.5 NEW CLINICAL RESEARCH STAFF – 100%, BUT NOT LESS THAN 5, OF ALL VISITS COMPLETED BY A NEW STAFF MEMBER WILL RECEIVE A PROMPT QA AUDIT UNTIL COMPETENCY IS DETERMINED.

3.3.2.1.6 SCREEN FAILURE/CONSENT WITHDRAWAL – ALL SCREEN FAILURES/CONSENT WITHDRAWAL RECORDS WILL BE REVIEWED TO ASSURE APPROPRIATE INFORMED CONSENT PROCESS AND DOCUMENTATION OF THE REASON FOR SCREEN FAILURE OR CONSENT WITHDRAWAL.

3.3.3 QA TOOLS

3.3.3.1 INTERNAL (SITE) SOURCES:

3.3.3.1.1 DMID CLINICAL QUALITY MANAGEMENT (CQMP): EMORY UNIVERSITY QUALITY ASSURANCE (QA) REVIEW TOOL (APPENDIX C). CHART REVIEW CHECKLISTS/WORKSHEETS ARE REVIEWED USING THIS TOOL.

3.3.3.1.1.1 INFORMATION COLLECTED AND DOCUMENTED ON APPENDIX D INCLUDE:

3.3.3.1.1.1.1 REVIEWER INITIALS

3.3.3.1.1.1.2 REVIEWER'S ROLE

3.3.3.1.1.1.3 REVIEWER DATE

3.3.3.1.1.1.4 PARTICIPANT IDENTIFICATION NUMBERS (PID) REVIEWED

3.3.3.1.1.1.5 SPECIFIC INDICATORS REVIEWED 3.3.3.1.1.1.6 FINDINGS/RESULTS OF REVIEW

3.3.3.1.1.1.7 PROTOCOL REGULATORY DOCUMENTS REVIEWED 3.3.3.1.1.1.8 TIME PERIOD COVERED BY THE REVIEW.

3.3.3.1.2 THE EMORY UNIVERSITY MONTHLY QC ERROR REPORT (APPENDIX D). INTERNAL QA/QC FINDINGS, SUMMARY REPORTS ARE REVIEWED AND REPORTED USING THIS TOOL.
3.3.3.1.3 THE EMORY UNIVERSITY REGULATORY FILE REVIEW TOOL (APPENDIX E) REVIEWED AND REPORTED USING THIS TOOL.
3.3.3.1.4 THE EMORY UNIVERSITY ANNUAL QUALITY MANAGEMENT REPORT (APPENDIX F)
3.3.3.1.5 THE EMORY UNIVERSITY VTEU SUMMARY REPORT LOG (APPENDIX G)

3.3.3.2 EXTERNAL SOURCES:
3.3.3.2.1 DATA ENTRY, QUERY/ERROR, OR TRANSMISSION REPORTS FROM THE DATA MANAGEMENT CENTER
3.3.3.2.2 CLINICAL SITE MONITORING REPORTS

THE ABOVE TOOLS WILL BE USED TO IDENTIFY TRENDS AND/OR AREAS OF CONCERN. THESE TRENDS WILL BE PRESENTED AND DISCUSSED WITH THE STAFF MEMBERS AT THE MONTHLY QM MEETINGS OR SOONER AS INDICATED. QUALITY SUMMARY REPORTS ARE REVIEWED BY THE SITE PI ON A MONTHLY BASIS. THE SIGNED REPORTS ARE KEPT IN A BINDER AND MADE AVAILABLE TO THE SITE MONITOR(S) UPON REQUEST.

3.3.4 THE MONTHLY QUALITY ASSURANCE REPORT (APPENDIX B) PROVIDES SITE-SPECIFIC SUMMARIES OF QM ACTIVITIES. THE RESULTS OF THESE ACTIVITIES ARE SHARED WITH THE SITE STAFF AT THE MONTHLY QM MEETING.

3.3.5 SITE MONITORING REPORTS, AS RECEIVED FROM EXTERNAL MONITORING GROUPS WILL ALSO BE UTILIZED AS A QA TOOL. THIS WILL ALLOW FOR THE REVIEW OF ANY TRENDS OR PROBLEMS IDENTIFIED BY THE EXTERNAL MONITOR. THESE REPORTS WILL BE GIVEN TO THE SITE STAFF UPON RECEIPT AND REVIEWED AND DISCUSSED AT THE MONTHLY QM MEETINGS AS INDICATED.

3.3.6 CORRECTIVE ACTION WILL BE TAKEN AS NEEDED TO ADDRESS AREAS OF CONCERN. SEE SECTION 9.5 CORRECTIVE AND PREVENTIVE ACTIONS

3.3.7 EVALUATION OF THE CORRECTIVE ACTION WILL TAKE PLACE AS PART OF THE ONGOING QA PROCESS. SEE SECTION 9.5 CORRECTIVE AND PREVENTIVE ACTIONS.

3.4 QUALITY ASSURANCE REPORTING REQUIREMENTS
3.4.1 QA FINDINGS WILL BE REPORTED USING THE EMORY UNIVERSITY QUALITY ASSURANCE (QA) REVIEW TOOL (APPENDIX A).
3.4.2 THE QA SUMMARY REPORT WILL INCLUDE IDENTIFICATION OF PROBLEMS, IDENTIFICATION OF POSSIBLE CAUSES, AND ANY CORRECTIVE AND PREVENTATIVE ACTIONS TAKEN.

3.4.3 IF AN UNREPORTED SAE IS IDENTIFIED DURING THE QM ACTIVITIES, THE EVENT WILL BE REPORTED PER PROTOCOL, DMID POLICY AND INSTITUTIONAL REQUIREMENTS

3.5 PROTOCOL-SPECIFIC CQMP:

3.5.1 A PROTOCOL-SPECIFIC CQMP WILL BE DEVELOPED FOR APPLICABLE PROTOCOLS PER PI DIRECTION.

3.5.2 A PROTOCOL-SPECIFIC CQMP MAY BE DEVELOPED AND IMPLEMENTED FOR PROTOCOLS BEING CONDUCTED AT MULTIPLE SITES WITHIN THE EMORY NETWORK, OR FOR EMORY SUBCONTRACTOR SITES INCLUDING DOMESTIC AND INTERNATIONAL LOCATIONS.

3.5.3 THE PROTOCOL-SPECIFIC CQMP TEMPLATE, AVAILABLE ON THE DMID CROMS WEBSITE, WILL BE UTILIZED AND WILL BE MODIFIED TO DEFINE AND PROVIDE DATA FOR PROTOCOL- DRIVEN PARAMETERS FOR QC AND QA RECORDING AND REPORTING.

3.5.4 QUALITY MANAGERS AT THE HOPE CLINIC AND/OR ECC WILL OVERSEE IMPLEMENTATION THE PROTOCOL-SPECIFIC CQMP AND WILL ENSURE THAT SITE STAFF ARE TRAINED IN PROPER CQMP USE AND PROCESS.

3.6 RETENTION OF QUALITY MANAGEMENT (QM) DOCUMENTS

3.6.1 THE CQMP WILL BE SIGNED AND DATED BY THE PI AND KEPT ON FILE.

3.6.2 COMPLETED QA SUMMARY REPORTS, CHART REVIEW TOOLS AND REGULATORY FILE REVIEW TOOLS WILL BE KEPT ON FILE AND ACCESSIBLE UPON DMID REQUEST.

3.7 OVERSIGHT OF SUBCONTRACTOR SITE(S), IF APPLICABLE:

3.7.1 SUBCONTRACTOR SITES WILL BE REQUIRED TO DEVELOP AND IMPLEMENT AN EMORY APPROVED CQMP UTILIZING A CONSISTENT CQMP FORMAT DEFINED BY EMORY.

3.7.2 THE PI, WITH THE ASSISTANCE OF THE QUALITY MANAGER, WILL WORK WITH THE SUBCONTRACTOR SITE TO DRAFT A PROTOCOL-SPECIFIC CQMP.

3.7.3 THE CQMP WILL BE COMPLETED BY THE EMORY SUBCONTRACTOR/PERFORMANCE SITES CONDUCTING DMID-FUNDED PROTOCOLS, AND WILL BE CONSISTENT WITH A PARTICULAR PROTOCOL.

3.7.4 IF QC AND QA TOOLS (AS DESCRIBED IN SECTIONS 3.2.4 AND 3.3.3) ARE USED BY SUBCONTRACTOR/PERFORMANCE SITES FOR DOCUMENTING QUALITY REVIEWS, PROCESSES AND INSTRUCTIONS WILL ENSURE CONSISTENT APPLICATION/IMPLEMENTATION ACROSS SITES.

3.7.5 IN THE CASE OF MULTI-SITE PROTOCOLS, A SINGLE CQMP MIGHT BE GENERATED FOR CONSISTENCY, AS DIRECTED BY THE DMID CPM.

3.7.6 THE CQMP WILL BE DEVELOPED, FINALIZED AND APPROVED BY EMORY, AND DMID AS APPLICABLE, PRIOR TO INITIATION OF THE PROTOCOL.

3.7.7 MONTHLY SUBCONTRACTOR REPORTS WILL BE SUBMITTED TO THE PRIME CONTRACT SITE BY THE SPECIFIED DUE DATE. REPORTS WILL INCLUDE: DOCUMENTATION OF ONGOING CQMP

ACTIVITIES, DESCRIPTION OF PROBLEMS IDENTIFIED, CORRECTIVE ACTION PLANS, IF REQUIRED, AND OTHER SPONSOR REQUIREMENTS/CONTRACTUAL OBLIGATIONS.

4.0 KEY QUALITY INDICATORS

THE KEY INDICATORS THAT WILL BE AUDITED IN EACH VOLUNTEER RECORD SELECTED FOR INTERNAL QA REVIEW ARE:

4.1 INFORMED CONSENT FORM AND PROCESS

4.2 ASSESSMENT OF UNDERSTANDING AS APPLICABLE

4.3 ELIGIBILITY CRITERIA AND PROCESS

4.4 STUDY PRODUCT MANAGEMENT: RECEIPT, STORAGE, PREPARATION, TRANSPORT, ADMINISTRATION, AND ACCOUNTABILITY (IF APPLICABLE) – PHARMACY QM HANDLED BY EU PHARMACY AND RECORDS ARE ON FILE.

4.4.1 REVIEW AND COMPARISON OF THE STUDY PRODUCT ACCOUNTABILITY LOGS, SHIPPING RECORDS, AND THE STUDY PRODUCT INVENTORY

4.4.2 MASKING PROCEDURES (MAINTENANCE OF STUDY BLIND, STUDY PERSONNEL RESTRICTIONS)

4.4.3 RANDOMIZATION CODE LIST AND DECODING PROCEDURES

4.4.4 STUDY PRODUCT STORAGE, HANDLING, AND LABELING PROCEDURES

4.4.5 VACCINE OR OTHER STUDY PRODUCT PREPARATION PROCEDURES

4.4.6 STUDY PRODUCT ADMINISTRATION PROCESSES

4.5 ADVERSE EVENTS (AE), SERIOUS ADVERSE EVENTS (SAE) IDENTIFICATION AND REPORTING AS APPLICABLE

4.6 PROTOCOL VISITS (EVALUATE FOR MISSED VISITS, OUT OF WINDOW VISITS, LOST TO FOLLOW-UP, ETC.)

4.7 PROTOCOL-SPECIFIC PROCEDURES (ALL INCLUSIVE)

4.8 INTERVENTION/STUDY DISCONTINUATION

4.9 REACTOGENICITY (IF APPLICABLE)

4.10 SPECIMENS -

4.10.1 PROCESSING SPECIMENS AS PER PROTOCOL AND/OR MANUAL OF OPERATIONAL PROCEDURES – QM HANDLED BY LABORATORY MANAGER

4.10.2 STORAGE OF SPECIMENS (REQUIRED CONDITIONS, LOCATION, LENGTH OF STORAGE) – QM HANDLED BY LABORATORY MANAGER.

4.10.3 DOCUMENTATION (SHIPPING LOGS, TEMPERATURE DEVIATION REPORTING, ACCREDITATIONS, EQUIPMENT CALIBRATION, COMMUNICATIONS TO STUDY TEAM/PI) – QM HANDLED BY LABORATORY MANAGER.

4.11 CONCOMITANT/PROHIBITED MEDICATIONS

4.12 PROTOCOL DEFINE ENDPOINT IDENTIFICATION AND REPORTABLE AS APPLICABLE

4.13 SOURCE DOCUMENTS, SIGNATURES, INITIALS, DATE(S) (SEE APPENDIX E: THE EMORY UNIVERSITY REGULATORY FILE REVIEW TOOL)

4.14 INVESTIGATOR FILE REVIEW DEFICIENCIES (SEE APPENDIX E: THE EMORY UNIVERSITY REGULATORY FILE REVIEW TOOL)

4.15 ADDITIONAL PROTOCOL-SPECIFIC INDICATORS, AS APPLICABLE.

4.16 LABORATORY AND PHARMACY FOLLOW THEIR OWN QM PROCEDURES. THESE PROCEDURES HAVE BEEN REVIEWED INTERNALLY AND MONITORED DURING SITE VISITS AS WELL AS FEDERAL AUDITS WITH NO CONCERNS/ISSUES.

5.0 REGULATORY FILE REVIEW

THE REGULATORY FILE REVIEW WILL ENSURE DOCUMENTS AS LISTED IN THE INTERNATIONAL CONFERENCE ON HARMONIZATION (ICH) GUIDELINES FOR GOOD CLINICAL PRACTICE (GCP); E6 (R2), SECTION 8, ESSENTIAL DOCUMENTS FOR THE CONDUCT OF A CLINICAL TRIAL ARE PRESENT. THE RESULTS OF THE REGULATORY FILE REVIEW WILL BE DISCUSSED AT THE MONTHLY QM MEETING OR SOONER AS INDICATED.

5.1 FREQUENCY OF REVIEW: THE REGULATORY FILE WILL BE REVIEWED AT THE BEGINNING OF A STUDY (PRIOR TO THE FIRST MONITORING VISIT) AND IMMEDIATELY PRIOR TO STUDY TERMINATION (PRIOR TO THE CLOSE OUT VISIT).

5.2 ONGOING REGULATORY REVIEW IS CONDUCTED DURING WEEKLY VTEU OPERATIONS MEETINGS WHERE ALL PENDING/APPROVED AMENDMENTS, CONTINUING REVIEWS AND REPORTABLE EVENTS ARE DISCUSSED AND STATUS UPDATES ARE GIVEN. REGULATORY CORE MEETINGS ARE ALSO HELD ON A WEEKLY BASIS AND PENDING AMENDMENTS, CRS AND REPORTABLE EVENTS ARE DISCUSSED AS WELL AS ANY PENDING ISSUES OR IRB QUESTIONS, ETC. WHEN IRB APPROVAL IS GIVEN, THE REGULATORY COORDINATOR SENDS AN EMAIL OUT TO ALL STUDY TEAM MEMBERS CONFIRMING THAT ALL REGULATORY DOCUMENTS HAVE BEEN FILED APPROPRIATELY, INFORMING THEM OF CHANGES TO THE STUDY AND WHETHER OR NOT PARTICIPANTS NEED RE-CONSENT. THE REGULATORY FILE MAY ALSO BE REVIEWED DURING THE COURSE OF THE STUDY OR WHEN A PROTOCOL IS REVISED, PER PI DISCRETION.

5.3 ESSENTIAL REGULATORY DOCUMENTS WILL BE UPLOADED TO THE SITE ESSENTIAL REGULATORY DOCUMENTS (SERD) PORTAL WITHIN THE NIAID CLINICAL RESEARCH MANAGEMENT SYSTEM (CRMS) AS REQUESTED, AS WELL AS THE REGULATORY FILE LOCATED ON THE EMORY UNIVERSITY SHARE DRIVE.

5.4 THE EMORY UNIVERSITY REGULATORY FILE REVIEW TOOL (APPENDIX E).

5.5 IDENTIFIED ERRORS WILL BE BROUGHT TO THE ATTENTION OF THE APPROPRIATE STAFF MEMBER FOR CORRECTION WITHIN 3 BUSINESS DAYS OF THE ERROR(S) BEING IDENTIFIED.

6.0 TOOLS AND CHECKLISTS

6.1 INTERNAL (SITE) SOURCES:

6.1.1 THE EMORY UNIVERSITY QUALITY ASSURANCE (QA) REVIEW TOOL (APPENDIX A)

6.1.2 THE EMORY UNIVERSITY QUALITY ASSURANCE (QA) SUMMARY REPORT (APPENDIX B)

6.1.3 THE EMORY UNIVERSITY QUALITY CONTROL REVIEW TOOL (APPENDIX C)

6.1.4 THE EMORY UNIVERSITY MONTHLY QUALITY CONTROL (QC) ERROR REPORT (APPENDIX D)

6.1.5 THE EMORY UNIVERSITY REGULATORY FILE REVIEW TOOL (APPENDIX E)

6.1.6 THE EMORY UNIVERSITY ANNUAL QUALITY MANAGEMENT REPORT (APPENDIX F)

6.1.7 THE EMORY UNIVERSITY STANDARD OPERATING PROCEDURES (SOP)

6.2 EXTERNAL SOURCES:

6.2.1 DATA ENTRY, QUERY/ERROR, OR TRANSMISSION REPORTS FROM THE DATA MANAGEMENT CENTER

6.2.2 CLINICAL SITE MONITORING REPORTS

7.0 STAFF TRAINING / QUALIFICATIONS

EMORY UNIVERSITY HAS AN ON-BOARDING AND GENERAL TRAINING PROCEDURE THAT HAS BEEN OUTLINED IN THE SOP . THE REGULATORY COORDINATOR MAINTAINS DOCUMENTATION OF ALL STAFF TRAINING AND ESSENTIAL DOCUMENTS. DOCUMENTS ARE REVIEWED BY THE QM MANAGER, OR DESIGNEE, AS PART OF THE REGULATORY DEPARTMENT AUDITS TO ENSURE STAFF IS QUALIFIED AND HAVE DEMONSTRATED COMPETENCY PER EMORY AND SPONSOR REQUIREMENTS.

7.1 INSTITUTION-SPECIFIC TRAINING:

7.1.1 HUMAN SUBJECTS PROTECTION (CITI)- EVERY THREE (3) YEARS

7.1.2 GOOD CLINICAL PRACTICE (CITI) – EVERY THREE (3) YEARS

7.1.3 KEY CONCEPTS FOR INVESTIGATORS COURSE (INVESTIGATORS ONLY)

7.1.4 INTRODUCTION TO CLINICAL RESEARCH AT EMORY (COORDINATORS ONLY) – REQUIRED OF STAFF WITH LESS THAN 5 YEARS RESEARCH EXPERIENCE

7.1.5 HIPAA TRAINING – EVERY THREE (3) YEARS

7.1.6 BLOOD BORNE PATHOGENS TRAINING – ANNUALLY

7.1.7 BIOSAFETY TRAINING – EVERY THREE (3) YEARS

7.1.8 CPR CERTIFICATION (CLINICAL STAFF) – EVERY TWO (2) YEARS

7.1.9 LABORATORY SAFETY TRAINING – ANNUALLY

7. 2.0 A.L.C.O.A.C PRINCIPLES (WHEN AND HOW APPLIED TO SOURCE DOCUMENT(S)) OVERVIEW – BI- ANNUALLY DURING NETWORK OPERATIONS MEETINGS.

7.2 PROTOCOL-SPECIFIC TRAINING:

7.2.1 AS DESIGNATED BY THE SPONSOR

7.3 DMID-SPECIFIC TRAINING:
7.3.1 HUMAN SUBJECTS PROTECTION – EVERY THREE (3) YEARS
7.3.2 GOOD CLINICAL PRACTICE – EVERY THREE (3) YEARS
7.3.3 DMID REGULATORY FILE DOCUMENT GUIDELINES - EVERY THREE (3) YEARS
7.3.4 DMID SOURCE DOCUMENTATION STANDARDS - EVERY THREE (3) YEARS
7.3.5 INVESTIGATOR RESPONSIBILITIES - EVERY THREE (3) YEARS
7.3.6 DMID STUDY PRODUCT MANAGEMENT - EVERY THREE (3) YEARS
7.3.7 NIH COMPUTER SECURITY AWARENESS - ANNUALLY

8.0 IMPLEMENTATION AND CONDUCT

8.1 PROTOCOL IMPLEMENTATION

THE EMORY VTEU AIMS AT IMPLEMENTING THE PROTOCOL IN A TIMELY MANNER WITH TYPICALLY 90 DAYS BETWEEN PROTOCOL DISTRIBUTION TO SITE-SPECIFIC PROTOCOL ACTIVATION.

8.2 ACTIVATION TO FIRST ENROLLMENT

THE EMORY VTEU AIMS AT ENROLLING SUBJECTS IN A TIMELY MANNER. TYPICALLY, THE FIRST SUBJECT WILL BE ENROLLED IN EARLY PHASE TRIALS WITHIN 2 WEEKS OF SITE PROTOCOL ACTIVATION AND PRODUCT AVAILABILITY AT THE SITE.

8.3 ENROLLMENT

THE EMORY VTEU AIMS AT REACHING ENROLLMENT TARGETS WITH >90% ENROLLED WITHIN THE ASSIGNED PERIOD IN EARLY PHASE TRIALS.

8.4 VISIT COMPLETION

THE EMORY VTEU AIMS AT ACHIEVING HIGH RETENTION RATES WITH >90% VISITS COMPLETED WITHIN THE ASSIGNED PERIOD IN EARLY PHASE TRIALS.

9.0 CLINICAL QUALITY MANAGEMENT REPORTING

9.1 THE HOPE CLINIC AND ECC-VRC, WILL MAKE AVAILABLE STUDY-SPECIFIC QUALITY REPORTS TO THE IDCRC UPON REQUEST.

9.2 TOOLS/FORMS USED TO DOCUMENT / SUMMARIZE QUALITY REVIEWS: THE EMORY UNIVERSITY QUALITY CONTROL REVIEW TOOL (APPENDIX C) WILL BE COMPLETED BY THE QM MANAGER OR DESIGNEE. THIS LOG IDENTIFIES AND TRACKS CATEGORIES OF CASE REPORT FORM (CRF) ERRORS.

9.3 IDENTIFICATION OF PROBLEM AREAS: INFORMATION GATHERED VIA APPENDIX C WILL BE AGGREGATED AND REPORTED TO THE SITE STAFF, USING THE MONTHLY QC ERROR REPORT (APPENDIX D) AT THE MONTHLY QM MEETING. THIS TOOL WILL ALSO BE USED TO IDENTIFY TRENDS AS WELL.

9.4 TREND ANALYSIS: CLINICAL QUERY REQUESTS WILL BE REVIEWED AND ANSWERED BY THE STUDY COORDINATOR, OR A DESIGNEE, OR REFERRED TO THE ATTENTION OF THE APPROPRIATE STAFF MEMBER FOR A RESPONSE. THE STUDY COORDINATOR, OR DESIGNEE, WILL PROVIDE A RESPONSE TO THE SPONSOR WITHIN THE REQUIRED TIMEFRAME INDICATED BY THE SPONSOR. THE STUDY COORDINATOR, OR DESIGNEE, WILL MAINTAIN COPIES OF QUERY RESPONSES IN THE STUDY FILES.

9.5 CORRECTIVE AND PREVENTIVE ACTION PLAN(S): A ROOT CAUSE ANALYSIS WILL BE COMPLETED BY THE PI/STUDY STAFF ALONG WITH INPUT FROM THE QM MANAGER. A CORRECTIVE AND PREVENTIVE ACTION (CAPA) PLAN WILL BE DEVELOPED TO MITIGATE THE ISSUE AND PREVENT FUTURE OCCURRENCES. THE ACTION PLAN WILL BE DETERMINED, IMPLEMENTED AND MONITORED BY THE PI FOR EFFECTIVENESS AND PROBLEM/ISSUE RESOLUTION. THESE ACTIONS MAY INCLUDE, BUT ARE NOT LIMITED TO, CHANGING A PROCESS OR FORM, TRAINING, OR REASSIGNING A TASK. ANY ADVERSE TREND WILL BE RE-EVALUATED TO ASSESS THE EFFECTIVENESS OF THE CORRECTIVE ACTION. TIMELINES FOR IMPLEMENTATION AND RE-EVALUATION WILL BE SET BASED ON THE TYPE/ACUITY OF PROBLEM. THE PLAN AND RESULTS OF RE-EVALUATION WILL ALSO BE COMMUNICATED TO THE STUDY STAFF AT THE MONTHLY QM MEETING AND DOCUMENTED IN THE MONTHLY QM MEETING MINUTES. THE HOPE CLINIC AND ECC-VRC WILL RESPOND TO FEEDBACK FROM THE IDCRC ABOUT QUALITY ISSUES REPORTED TO THE SITE (E.G. THROUGH CROMS MONITORING OR FROM OTHER OVERSIGHT ACTIVITIES). SUCH RESPONSES MAY INCLUDE THE NEED TO PRODUCE FORMAL CAPAS.

9.6 REVISION TO THE CQMP: REVIEW AND/OR REVISION TO THE CQMP WILL BE PERFORMED BY THE VTEU CONTACT PI, VTEU PROJECT MANAGER AND QM STAFF ON AN ANNUAL BASIS OR SOONER IF INDICATED.

10.0 SITE EVALUATION OF THE CLINICAL QUALITY MANAGEMENT PLAN

10.1 CQMP REVIEW: THE EMORY UNIVERSITY QUALITY MANAGEMENT PLAN IS REVIEWED ANNUALLY. THE VTEU CONTACT PI, VTEU PROJECT MANAGER, AND THE QUALITY MANAGER, WITH INPUT FROM THE CLINICAL STAFF, WILL DETERMINE IF ANY REVISIONS ARE TO BE MADE TO THE QM PLAN. IF REVISIONS ARE MADE, ALL STAFF WILL BE TRAINED IN THE NEW PROCEDURE AND SUCH TRAINING WILL BE DOCUMENTED IN THE QM MEETING MINUTES.

10.2 ADMINISTRATIVE CHANGES TO THE CQMP, WHICH DO NOT IMPACT THE EFFECTIVENESS OF THE CQMP, WILL NOT REQUIRE A RE-REVIEW BY THE DMID-CROMS REVIEW TEAM. HOWEVER, THE SITE WILL PROVIDE REVISED DOCUMENTS TO THE DMID-CROMS CQMP REVIEW TEAM.

10.3. THE EMORY VTEU SUMMARY REPORT LOG (APPENDIX G) WILL BE KEPT IN A BINDER AND MADE AVAILABLE TO THE SITE MONITOR(S) UPON REQUEST.