STUDY TITLE: Effect of Antimalarial Drugs on the Immune Response to Rabies Vaccine for Post-exposure Prophylaxis. A Randomized, Open Label, Trial in Healthy US Adults Age 18-60 Years

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Effect of Antimalarial Drugs on the Immune Response to Rabies Vaccine for Post-exposure Prophylaxis. A Randomized, Open Label, Trial in Healthy US Adults Age 18-60 Years

Short Title: MALRAB

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Licensed Products: RabAvert® Rabies Vaccine
Chloroquine
Malarone
Doxycycline

Version Number: 11.0

09 May 2018

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US Federal regulations and ICH guidelines.

Principal Investigator:

Signed: _____ Date: _____

Name: Timothy P. Endy, MD, MPH

Title: Principal Investigator

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LIST OF ABBREVIATIONS

ACIP	Advisory Committee on Immunization Practices
AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CGHATS	Center for Global Health and Translational Science
CRU	Clinical Research Unit
DHHS	Department of Health and Human Services
eCRF	Electronic Case Report Form
EDC	Electronic data collection
FDA	Food and Drug Administration
FWA	Federalwide Assurance
GCP	Good Clinical Practice
GMT	Geometric Mean Titer
HDCV	Human diploid cell vaccine
HIV	Human Immunodeficiency virus
HRIG	Human Rabies Immunoglobulin
HRPO	Human Research Protection Office
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ID	Intradermal
IM	Intramuscular
IRB	Institutional Review Board
mL	milliliter
NIH	National Institute of Health
OHRP	Office for Human Research Protections
PBMC	Peripheral Blood Mononuclear Cell
PCECV	Purified chick embryo cell vaccine
PEP	Post exposure prophylaxis
PO	per os (oral)
PP	Per protocol
PI	Principal Investigator
QA	Quality Assurance
QAIP	Quality Assessment and Improvement Programs

QC	Quality Control
RFFIT	Rapid fluorescent foci inhibition test
RVNA	Rabies virus neutralization assay
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
SSP	Study Specific Procedure
SUNY	State University of New York
UMU	Upstate Medical University
US	United States
USAMRMC	United States Army Medical Research and Materiel Command
WFI	Water For Injection
WHO	World Health Organization

SYNOPSIS

Trial Title	Effect of Antimalarial Drugs on the Immune Response to Rabies Vaccine for Post-exposure Prophylaxis. A Randomized, Open Label, Trial in US Adults Age 18-60 Years
Short title	MALRAB
Clinical Phase	Investigational
Trial Site	State University of New York, Upstate Medical University, Center for Global Health and Translational Science
Principal Investigator	Timothy P. Endy, MD, MPH
Licensed Products	RabAvert® Rabies Vaccine Chloroquine Tablets Malarone Tablets Doxycycline Tablets
Planned Trial Period	September 2016 – August 2018
Trial Participants	18-60 year old adults living in the US
Planned Sample Size	100 vaccinated, 25 vaccinated subjects in each group
Treatment duration	1 month
Follow up duration	1 month
Trial Design	Randomized, open-label study with 100 vaccinated adults age ≥ 18 and ≤ 60 years at time of enrollment. After screening, eligible subjects will be enrolled and randomized into one of four groups and assigned a unique subject ID number. Each subject will be scheduled to receive four doses of rabies vaccine intramuscularly. Groups 1, 2 and 3 will receive 14 days of malaria chemoprophylaxis prior to first immunization. Group 4 will not receive malaria chemoprophylaxis and will receive first immunization on day 0. Blood and urine samples for research assays will be drawn at different time points throughout the study, depending on group assignment. As of April 30 2018, urine will not be collected and saved for transport to Syracuse University for those in group 1. This portion of the study is complete. Additional blood samples may be drawn if required to assess AEs and SAEs.

<table>
<tr><td></td><td>Schedule of Vaccine Administration
<table>
<tr><th>Day</th><th>0</th><th>3</th><th>7</th><th>14</th><th>17</th><th>21</th><th>28</th></tr>
<tr><td>Group 1</td><td></td><td></td><td></td><td>IM</td><td>IM</td><td>IM</td><td>IM</td></tr>
<tr><td>Group 2</td><td></td><td></td><td></td><td>IM</td><td>IM</td><td>IM</td><td>IM</td></tr>
<tr><td>Group 3</td><td></td><td></td><td></td><td>IM</td><td>IM</td><td>IM</td><td>IM</td></tr>
<tr><td>Group 4</td><td>IM</td><td>IM</td><td>IM</td><td>IM</td><td></td><td></td><td></td></tr>
</table></td></tr>
<tr><td>Inclusion Criteria:</td><td>Subjects must meet all of the following criteria in order to be eligible for trial enrollment:
1. Provide signed and dated informed consent form
2. Willing to comply with all study procedures and be available for the duration of the study
3. Male or female, aged ≥ 18 to ≤ 60 years on day of inclusion
4. In good general health based on medical history and physical exam</td></tr>
<tr><td>Exclusion Criteria:</td><td>A subject fulfilling any of the following criteria will be excluded from trial enrollment:
1. Subject is pregnant, or lactating, or of childbearing potential (to be considered of non-childbearing potential, a female must be post-menopausal for at least 1 year, surgically sterile, or using an effective method of contraception or abstinence from at least 4 weeks prior to the first vaccination and until at least 4 weeks after the last vaccination.
2. Participation in the 4 weeks preceding the first trial vaccination, or planned participation during the present trial period, in another clinical trial investigating a vaccine, drug, medical device, or medical procedure.
3. Previous history of receiving the rabies vaccine.
4. Previous history of receiving rabies immune globulin.
5. Any major psychiatric disorder, such as severe depression, severe anxiety disorder, psychosis, schizophrenia, other major psychiatric disorders, or seizures. History of mild depression or anxiety disorder that are well controlled are not exclusion criteria.
6. Use of any immunosuppressive drug at the time of the study or 30 days previously. Topical steroids will not be considered an immunosuppressive drug and their use will not be considered an exclusion criteria.
7. Any immunosuppressive disorder, such as HIV infection, common variable immunodeficiency, active cancers or chemotherapy.
8. History of renal insufficiency or requiring dialysis.
9. Have any condition that would, in the opinion of the site investigator,</td></tr>
</table>

	place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol. 10. Previous adverse reaction to any of the antimalarial drugs used in this study. Temporary Exclusion Criteria: Moderate or severe acute illness/infection (according to investigator judgment) or febrile illness (temperature ≥ 38.0°C [≥ 100.4°F) on the day 0. A prospective subject should not be included in the study until the condition has resolved or the febrile event has subsided. If the delay for the febrile illness exceeds the window between screening and Day 0, or if deemed necessary by the investigator, a prospective subject may be re-screened once the fever has resolved. Recent or scheduled receipt of any vaccine 4 weeks prior to day 0.
Randomization:	Randomization will be performed through a computer generated process. A standard permuted-block design will be used for randomization/allocation in order to avoid undesirable differences in the numbers of patients assigned to each group. The randomization schedule will be generated by our statistician using SAS 9.3 PROC PLAN. Variable block sizes will be kept confidential from the protocol to reduce the predictability of the treatment assignments. Subjects who withdraw prior to first vaccination will be replaced.
Licensed Product, Formulation, Dose, Route of Administration	RabAvert® rabies vaccine (Novartis): 1.0 mL reconstituted freeze dried vaccine containing ≥ 2.5 international units of rabies antigen administered intramuscularly (IM). Chloroquine: Chloroquine phosphate two 250 mg tablets containing 150 mg base administered weekly, orally. Malarone: 250 mg Malarone tablet containing 250 mg atovaquone and 100 mg proquanil hydrochloride administered daily, orally. Doxycycline: 100 mg tablet administered daily, orally.
Objectives:	**Primary:** Compare GMT at 14 days post completion of four dose PEP with PCECV in each of the malaria prophylaxis groups with control to determine if a fifth dose of PEP at that point would be of any added value. **Secondary:** To evaluate GMT over protective titer prior to third dose, fourth dose and 28 days post fourth dose of PCECV. **Exploratory:** Cell Mediated Immunity, Proteomics if differences in the immune responses are seen, Urine analysis in chloroquine group.
Endpoints:	**Primary:** GMT by RFFIT at 14 days post completion of four doses PEP

	in prophylaxis groups vs. control group **Secondary:** GMT by RFFIT prior to third or fourth dose of rabies vaccine and 28 days post fourth vaccine in prophylaxis groups vs control group. **Exploratory:** Cell Mediated Immunity, Proteomics if differences in the immune response are seen, Urine analysis in chloroquine group.
Statistical Methods:	This will be a descriptive study to assess immunogenicity of a licensed rabies vaccine using recommended dosage schedules in the presence of malaria chemoprophylaxis. No formal statistical hypothesis tests will be conducted. Descriptive analyses will be based on the per-protocol analysis sets. Exploratory statistical analyses of final data may be conducted, if indicated by the descriptive results. Parametric, non-parametric and resampling (bootstrapping) methods for statistical inference may be used in exploratory analyses, based on data compliance with assumptions of methods. P -values ≤ 0.05 will be considered significant and p-values ≤ 0.10 will be considered a trend. Confidence intervals will be constructed at α=0.05 and α=0.10. When necessary, p-value corrections for multiple comparisons will be applied. Descriptive and any inferential analyses will be carried out using SAS Version 9.2 (or higher), which is licensed and supplied by SAS Institute, Cary, NC, USA.

1 KEY ROLES AND CONTACT INFORMATION

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Rabies is present on all continents and enzootic in most African and Asian countries where U.S. military personnel deploy, including countries where malaria is also endemic and where U.S. military personnel are required to take malaria prophylaxis. (1). The incubation period for rabies can be as long as 10 years following infection, placing soldiers who have been exposed, but not rabies vaccinated, at risk for a prolonged period of time. Current DOD policy does not require routine vaccination prior to deployment in rabies enzootic countries. The death of a U.S. soldier in 2011 prompted a survey of other soldiers deployed at the same time. It was determined that 140 out of 8,600 screened had exposures for which post-exposure prophylaxis was indicated but did not seek medical attention (2).

Antimalarial drugs, chloroquine in particular, have previously been shown to impair the immune response to intradermal (ID) rabies vaccination. A randomized controlled trial was performed in veterinary students to evaluate the antibody response to ID HDCV pre-exposure prophylaxis administered with and without chloroquine (3). The study consisted of 51 students not previously vaccinated against rabies, of which 26 received 300mg chloroquine base per week and 25 did not. All received 0.1 mL of rabies vaccine ID on days 0, 7, and 28. Chloroquine was given weekly to the treatment group starting 9 days before the first dose of vaccine and continued until day 48. Rabies-neutralizing antibody titers for the chloroquine group were significantly lower than for the control group on days 28, 49, and 105 post-vaccination, indicating that chloroquine taken in pre-exposure prophylaxis doses reduces the antibody response to primary immunization with ID HDCV. This observation was also reflected in a Peace Corp population in Thailand who had received chloroquine and had reduced immune responses to rabies vaccine (4). As a result, current ACIP guidelines recommend that exposed persons who are taking malaria prophylaxis should receive a fifth dose of rabies vaccine 28 days after the exposure. These guidelines do not differentiate between drugs used for malaria prophylaxis (5).

Obtaining rabies vaccine and rabies immune globulin in a deployed setting can be challenging (6). A full understanding of the requirements for protecting exposed individuals is necessary for appropriate decision making in a resource-constrained environment.

2.2 Rationale

This is an exploratory trial to evaluate the effect of antimalarial drugs on the immune response generated by rabies vaccine when administered for post-exposure prophylaxis. This study will use the FDA approved post-exposure prophylaxis vaccine regimen (without rabies immune globulin) in the presence or absence of an FDA-approved malaria chemoprophylaxis regimen.

Rabies post-exposure prophylaxis in unvaccinated individuals who are not on malaria prophylaxis consists of four, 1.0-mL IM injections of the human diploid cell vaccine (HDCV) or purified chick embryo cell (PCECV) rabies vaccine on days 0, 3, 7, and 14 (5). In the event of an exposure, human rabies immunoglobulin (HRIG) would also be administered on day 0, at 20 units per kg of body weight, IM, preferably at the site of exposure and not in the same location as the first vaccine dose. In previously vaccinated individuals, HRIG is not administered, and two doses of vaccine are given, one at day 0 and one at day 3. Current ACIP guidelines recommend that because "corticosteroids, other immunosuppressive agents, antimalarials, and immunosuppressive illnesses might reduce immune responses to rabies vaccines substantially," exposed persons on antimalarials should receive a fifth dose of rabies vaccine on day 28. The guidelines do not differentiate between malaria prophylaxis regimens (5), therefore this fifth dose is indicated in all compliant soldiers serving in malaria-endemic areas.

This study will administer the post-exposure regimen to volunteers from a US population of military age who are taking one of three malaria prophylaxis regimens or no malaria prophylaxis. The primary endpoint is the rabies titer 28 days after the first dose or 14 days after completion of the 4-dose regimen given to individuals not on malaria prophylaxis (ie, the time point at which the fifth dose would be given) to determine if there is any added value in administering this fifth dose.

Malarone and doxycycline were chosen because they are the drugs most commonly prescribed to deploying soldiers for malaria prophylaxis. Chloroquine was chosen because this was the drug previously shown to affect the immune response to intradermal rabies vaccination given for pre-exposure prophylaxis (3). Its effect on the immune response to intramuscular rabies vaccines given in the regimen for post-exposure prophylaxis has not previously been studied.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

Subjects receiving Rabies Vaccine

Reactions to the current rabies vaccine are mild with local pain, erythema and swelling at the injection site. Systemic reactions are less frequent and include headache and malaise (1). Guillain-Barré syndrome has been reported as a rare event following vaccination but its association with vaccination is uncertain (1).

Subjects receiving Chloroquine

Reactions to chloroquine include gastrointestinal upset, headache and pruritus, which may be intolerable, is common among Africans.

Subjects receiving Malarone

Reactions to malarone are nausea, vomiting, abdominal pain, headache, diarrhea, weakness, loss of appetite, and dizziness.

Subjects receiving Doxycycline

Reactions to doxycycline are mild nausea, mild diarrhea, gastrointestinal upset, mild skin rash, photosensitivity and itching and for females, vaginal itching or discharge. This medication may also have more severe symptoms such as headaches, fever, chills, severe diarrhea, and decreased urination.

All subjects

Potential risks of blood sampling include tenderness or bruising at the spot where blood was drawn. Fainting or dizziness can occur after a blood draw, but this is uncommon.

2.3.2 Potential Benefits

Potential benefits to subjects receiving the rabies vaccine include potentially developing protective antibody levels to rabies.

There are no potential benefits to malaria chemoprophylaxis for this study.

3 OBJECTIVES AND ENDPOINTS

3.1 Study Objectives

3.1.1 Primary Study Objective

To compare Geometric Mean Titer (GMT) at 14 days post completion of four dose post exposure prophylaxis (PEP) with PCECV in each of the malaria prophylaxis groups with control to determine if a fifth dose of PEP at that point would be of any added value.

3.1.2 Secondary Study Objective

To evaluate GMT over protective titer prior to third dose and fourth dose and 28 days post fourth dose of PCECV.

3.1.3 Exploratory Study Objective

To explore Cell Mediated Immunity and Proteomics if differences in the immune responses are seen. To explore the hypothesis that a lysosomal protein saposin B may play a role in mitigating chloroquine toxicity and/or may be a source of toxicity associated with long term chloroquine use.

3.2 Study Endpoints

3.2.1 Primary Endpoint (Immunogenicity)

GMT by RFFIT at 14 days post completion of four doses PEP in prophylaxis groups vs. control group.

3.2.2 Secondary Endpoint (Immunogenicity)

GMT by RFFIT prior to third or fourth dose of rabies vaccine and 28 days post fourth vaccine in prophylaxis groups vs control group.

3.2.3 Exploratory

To explore Cell Mediated Immunity and Proteomics if differences in the immune responses are seen. Urine analysis to explore the hypothesis that a lysosomal protein saposin B may play a role in mitigating chloroquine toxicity and/or may be a source of toxicity associated with long term chloroquine use.

4 STUDY DESIGN

This is a randomized, open-label, single site study conducted in 100 vaccinated healthy adults age ≥ 18 to ≤ 60 years old in the US.

Subjects meeting inclusion/exclusion criteria will be randomly assigned to one of the four groups through a computer generated process.

Rabies vaccine will be administered to subjects who have been randomized into four groups. Group 1 will receive Chloroquine (po) for 14 days prior to first rabies vaccination. Group 2 will receive Malarone (po) for 14 days prior to first rabies vaccination. Group 3 will receive Doxycycline (po) for 14 days prior to first rabies vaccination. Group 4 (control) will begin rabies vaccination without antimalarial prophylaxis. See Table 1 for group allocation and number of subjects per group

Table 1. Schedule of vaccine administration and subjects per group

Day	**0**	**3**	**7**	**14**	**17**	**21**	**28**	**Number of subjects**
Group 1 Chloroquine				IM	IM	IM	IM	25
Group 2 Malarone				IM	IM	IM	IM	25
Group 3 Doxycycline				IM	IM	IM	IM	25
Group 4 Controls	IM	IM	IM	IM				25

Blood samples will be taken at most visits for RFFIT assays. Samples will also be taken or exploratory proteomic and cellular assays.

In all groups additional blood samples may be taken if required to assess AEs. Additional biological samples (e.g., body fluid, tissue samples) may be collected in the event of an AE, or SAE.

Clinical site personnel will record immediate unsolicited AEs that occur within 30 minutes after receiving the rabies vaccine. The occurrence of SAEs will be collected throughout the study.

Expected study duration for Groups 1-3 once enrolled in the study is approximately 2 months. Expected study duration for Groups 4 once enrolled in the study is approximately 1 month.

5 STUDY ENROLLMENT AND WITHDRAWAL

Healthy subjects will be recruited from Syracuse and the surrounding area for participation in this clinical trial.

Enrollment will continue until 25 subjects are vaccinated in each group. Any subject withdrawing prior to first vaccination will be replaced. Any subject withdrawing after first vaccination will not be replaced.

Subjects will be compensated at each study visit. Specifics of compensation is included in the informed consent and approved by the Upstate IRB.

5.1 Subject Inclusion Criteria

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

1. Provide signed and dated informed consent form.
2. Willing to comply with all study procedures and be available for the duration of the study.
3. Male or female, aged ≥ 18 to ≤ 60 years on day of inclusion.
4. In good general health based on medical history and physical exam.

5.2 Subject Exclusion Criteria

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

1. Subject is pregnant, or lactating, or of childbearing potential (to be considered of non-childbearing potential, a female must be post-menopausal for at least 1 year, surgically sterile, or using an effective method of contraception or abstinence from at least 4 weeks prior to the first vaccination and until at least 4 weeks after the last vaccination.
2. Participation in the 4 weeks preceding the first trial vaccination, or planned participation during the present trial period, in another clinical trial investigating a vaccine, drug, medical device, or medical procedure.
3. Previous history of receiving the rabies vaccine.
4. Previous history of receiving rabies immune globulin.
5. Any major psychiatric disorder, such as severe depression, severe anxiety disorder, psychosis, schizophrenia, other major psychiatric disorders, or seizures. History of mild depression or anxiety disorder that are well controlled are not exclusion criteria.

6. Use of any immunosuppressive drug at the time of the study or 30 days previously. Topical steroids will not be considered an immunosuppressive drug and their use will not be considered an exclusion criteria.

7. Any immunosuppressive disorder, such as HIV infection, common variable immunodeficiency, active cancers or chemotherapy.

8. History of renal insufficiency or requiring dialysis.

9. Have any condition that would, in the opinion of the site investigator, place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol.

10. Previous adverse reaction to any of the antimalarial drugs used in this study.

Temporary Exclusion Criteria: Moderate or severe acute illness/infection (according to investigator judgment) or febrile illness (temperature ≥ 38.0°C [≥ 100.4°F]) on day 0.. A prospective subject should not be included in the study until the condition has resolved or the febrile event has subsided. If the delay for the febrile illness exceeds the window between screening and vaccination, or if deemed necessary by the investigator, a prospective subject may be re-screened once the fever has resolved.

Recent or scheduled receipt of any vaccine 4 weeks prior to day 0.

5.3 Strategies for Recruitment and Retention

Subjects will be recruited via IRB approved posters, email, radio advertisements, social media outlets, word of mouth, or recruiting agency.

5.4 Treatment Assignment Procedures

Randomization and analysis will be performed by Upstate Medical University.

Randomization will be performed through a computer generated process. A standard permuted-block design will be used for randomization/allocation in order to avoid undesirable differences in the numbers of patients assigned to each group.

5.4.1 Randomization Procedures

The randomization schedule will be generated by the statistician using SAS 9.3 PROC PLAN. Variable block sizes will be kept confidential from the clinical staff to reduce the predictability of the treatment assignments. Subjects who withdraw prior to first vaccination will be replaced by the next enrolled subject who will be assigned to the same group as the withdrawn subject.

The first 39 subjects would be randomized to malarone, doxycycline and control group (1:1:1). Subjects 40-100 would be randomized to chloroquine, malarone, doxycycline and control groups (2:1:1:1). This would ensure that upon completion of enrollment

subjects would be randomly allocated 1:1:1:1 across all four groups - three treatment groups and one control group.

5.5 Subject Withdrawal

Subjects are free to withdraw from participation in the study at any time upon request.

5.5.1 Contraindications for Subsequent Vaccinations

5.5.1.1 Temporary Contraindications

An investigator may temporarily postpone a subject's further vaccination until the following are resolved:

- Ongoing clinical AE or biological abnormality related to the trial vaccination
- Febrile illness (temperature ≥ 38.0°C [≥ 100.4°F]) or moderate or severe acute illness/infection on the day of vaccination, according to Investigator judgment.
- Acute illness on day of vaccination that the investigator determines the subject to be ineligible for vaccine that day.

5.5.1.2 Definitive Contraindications

An investigator may terminate a study subject's participation in the study if the following contraindications are met:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- Significant noncompliance with the protocol, based on the Investigator's judgment.

5.5.2 Handling of Subject Withdrawals or Subject Discontinuation of Study Intervention

Subjects who withdraw or are withdrawn from the study will have the reason clearly documented in their source records and on the corresponding eCRF.

In the case of subjects who fail to return for a follow-up examination, documented reasonable effort (i.e., documented telephone calls) should be undertaken to locate or recall them, or at least to determine their health status while fully respecting their rights. These efforts should be documented in the source documents.

Subjects who have received at least one dose of vaccine will not be replaced. If a subject has already received at least one dose of the study vaccine and experiences a safety related contraindication to further participation in the trial, they will continue to be followed for safety evaluation for two weeks after the last vaccination was given or the time of withdrawal, whichever is later. This safety evaluation will include targeted physical exam if deemed necessary by the physician, injection site assessment (if within 2 weeks of last vaccination), and blood draw (if within two weeks of last vaccination).

5.5.3 Classification of Subjects Who Discontinue the Study

For any subject who discontinues the trial prior to completion, the most serious reason for early termination will be checked in the eCRF. Reasons will be listed from the most serious to the least serious as follows:

- **Serious adverse event:** To be used when a subject drops out of or is withdrawn from the study by the Investigator because of the occurrence of an SAE, as defined in *Section 9.1.3*.
- **Other adverse event:** To be used when a subject drops out of or is withdrawn from the study by the Investigator because of the occurrence of an AE other than an SAE, as defined in *Section 9.4.1.5.*
- **Non-compliance with protocol:** To be used when the Investigator withdraws a subject from the study because of failure to follow protocol guidelines (e.g., not attending visits, not being available for telephone calls, not providing blood samples). This termination category may also be used if it is retrospectively discovered that a subject did not fulfill the eligibility criteria. The Investigator will provide a comment as to the specific cause of non-compliance.
- **Lost to follow-up:** To be used when the Investigator withdraws a subject from the study because of failure to establish contact, as outlined in *Section 5.2.9.* The Investigator will provide documentation that contact was attempted (i.e., return of unsigned certified letter receipt).
- **Voluntary withdrawal not due to an adverse event:** To be used when a subject drops out of the study for any reason other than those listed above.

5.6 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to principal investigator or funding agency. If the study is prematurely terminated or suspended, the principal investigator will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

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

- Determination of unexpected, significant, or unacceptable risk to subjects.

- Insufficient adherence to protocol requirements.
- Data are not sufficiently complete and/or evaluable.

6 STUDY INTERVENTION

6.1 Study Product Description

There are four (4) products used for this study: RabAvert® (rabies vaccine) produced by Novartis Vaccines and Diagnostics GmbH, chloroquine phosphate, malarone and doxycycline hyclate.

RabAvert® (Rabies Vaccine)

RabAvert® (rabies vaccine) produced by Novartis Vaccines and Diagnostics GmbH is a sterile freeze-dried vaccine obtained by growing the fixed-virus strain Flury LEP in primary cultures of chicken fibroblasts. The strain Flury LEP was obtained from American Type Culture Collection as the 59th egg passage. The growth medium for propagation of the virus is a synthetic cell culture medium with the addition of human albumin, polygeline (processed bovine gelatin) and antibiotics. The virus is inactivated with β-propiolactone, and further processed by zonal centrifugation in sucrose density-gradient. The vaccine is lyophilized after addition of a stabilizer solution that consists of buffered polygeline and potassium glutamate. One dose of reconstituted vaccine contains less than 12 mg polygeline (processed bovine gelatin), less than 0.3 mg human serum albumin, 1 mg potassium glutamate and 0.3 mg sodium EDTA. Small quantities of bovine serum are used in the cell culture process. Bovine components originate only from the United States, Australia and New Zealand. Minimal amounts of chicken protein may be present in the final product; ovalbumin content is less than 3 ng/dose (1 mL), based on ELISA. In the final vaccine, neomycin is present at < 1 µg, chlortetracycline at < 20 ng, and amphotericin B at < 2 ng per dose. RabAvert® is intended for intramuscular (IM) injection. The vaccine contains no preservative and should be used immediately after reconstitution with the supplied Sterile Diluent for RabAvert® (Water for Injection). The potency of the final product is determined by the US National Institute of Health (NIH) mouse potency test using the US reference standard. The potency of one dose (1.0 mL) RabAvert® is at least 2.5 IU of rabies antigen. RabAvert® is a white, freeze-dried vaccine for reconstitution with the water for injection diluent prior to use; the reconstituted vaccine is a clear to slightly opaque, colorless solution.

Form: Powder and solvent for suspension for injection
Dose: 1.0 mL of the reconstituted vaccine
Route: IM
Lot number: 532011A

Chloroquine Phosphate Tablets

Chloroquine Phosphate Tablets are an antimalarial and amebicidal drug. Each tablet, for oral administration, contains 250 mg chloroquine phosphate (equivalent to 150 mg base). Inactive ingredients 125 mg: Calcium Stearate, Colloidal Silicon Dioxide, Dibasic Calcium Phosphate, Microcrystalline Cellulose, and Talc. Inactive ingredients 250 mg:

Colloidal Silicon Dioxide, Corn Starch, Lactose Monohydrate, Magnesium Stearate, Microcrystalline Cellulose, Polyvinylpyrrolidone, Sodium Starch Glycolate, and Titanium Dioxide. Film Coating and Polishing Solution contains: D&C Red #27 Aluminum Lake, D&C Yellow #10 Aluminum Lake, FD&C Blue #1 Aluminum Lake, Hypromellose, Polyethylene Glycol, Polysorbate 80 and Titanium Dioxide.

Malarone

Malarone is an antimalarial drug. Each tablet, for oral administration, contains 250 mg atovaquone and 100 mg proguanil hydrochloride. The inactive ingredients are low-substituted hydroxypropyl cellulose, magnesium stearate, microcrystalline cellulose, poloxamer 188, povidone K30, and sodium starch glycolate. The tablet coating contains hypromellose, polyethylene glycol 400, polyethylene glycol 8000, red iron oxide, and titanium dioxide.

Doxycycline hyclate

Doxycycline hyclate is a tetracycline antibiotic. Each tablet, for oral administration, contains specially coated pellets of doxycycline hyclate equivalent to 100 mg of doxycycline. Doxycycline hyclate is a yellow crystalline powder soluble in water and in solutions of alkali hydroxides and carbonates. Doxycycline has a high degree of lipid solubility and a low affinity for calcium binding. It is highly stable in normal human serum. Doxycycline will not degrade into an epianhydro form. Inert ingredients in the tablet formulation are: lactose monohydrate; microcrystalline cellulose; sodium lauryl sulfate; sodium chloride; talc; anhydrous lactose; corn starch; crospovidone; magnesium stearate; cellulosic polymer coating.

6.1.1 Acquisition

Medication will be purchased by the study team through approved vendors and shipped directly to Upstate University Hospital Pharmacy.

6.1.2 Packaging

RabAvert® comes in individually packaged unit doses for IM administration.
Chloroquine USP 250 mg is a pink, round tablet.
Malarone is a pink, film-coated, round, biconvex tablet.
Doxycycline is a white, oval, scored tablet.

Subjects in the chloroquine group will be dispensed a 1 day supply of antimalarial. All doses will be taken weekly during the weekly study visit. Subjects in the malarone and doxycycline groups will be dispensed a 42 day supply of antimalarial. The first dose will be taken during the first study visit, the remaining doses will be taken daily by the subject.

6.1.3 Product Storage and Stability

The Investigator is responsible for product management or will designate a staff member to assume this responsibility.

Vaccine will be stored at SUNY-UMU Clinical Research Unit in a secure location with restricted access. Vaccine will be stored in a refrigerator at a temperature ranging from +2°C to +8°C. The vaccine must not be frozen. The temperature must be monitored and documented for the entire time that the vaccine is at the trial site. In case of accidental freezing or disruption of the cold chain, vaccine must not be administered and must be quarantined, and the Investigator or authorized designee should contact the Novartis representative for further instructions. Medication in the form of tablets will be stored at room temperature in a secure location within Upstate University Hospital Pharmacy.

6.2 Accountability Procedures for the Study Product

Vaccine and medication accountability will be the responsibility of the Investigator or designee, who will receive and distribute product and maintain accountability logs for all products.

As all products used in this study are licensed products, any undispensed product may be used as directed by the PI for other studies.

6.3 Assessment of Subject Compliance with Study Product Administration

All subjects will be administered RabAvert® by study staff. Chloroquine will be administered to subjects by direct observation by study staff.

Subjects receiving malarone or doxycycline will be asked to return any unused portion of the product at the next study visit. Any unused doses will be documented by the site staff and destroyed by the monitor after accountability. Any dispensed doses that are lost by subjects will be thoroughly documented and replaced.

6.4 Concomitant Medications

Use of any immunosuppressive drug at the time of the study or 30 days previously.

6.5 Preparation and Administration of Product(s)

RabAvert® rabies vaccine is reconstituted with 1 mL Water for Injection (WFI) and 1 mL is administered by IM injection in the deltoid muscle by CRU nurses. All subjects will receive four vaccinations. Schedule will be determined by group assignment.

Chloroquine, Malarone and Doxycycline are oral administration tablets.

The dose for chloroquine will be 500 mg po for once weekly administration for 6 weeks.

The dose for malarone will be 250/100 mg po once daily administration for 42 days.

The dose for doxycycline will be 100 mg po for once daily administration for 42 days.

See *Appendix A* and *Appendix B* for Study Events and *Appendix C* for Blood Draw Schedule.

7 STUDY SCHEDULE AND PROCEDURES

7.1 Informed Consent Procedures

Informed consent is the process by which a subject voluntarily confirms his or her willingness to participate in a particular trial. Informed consent must be obtained before any study procedures are performed. The process is documented by means of a written, signed, and dated ICF. In accordance with GCP, prior to signing and dating the consent form, the subject must be informed by appropriate study personnel about all aspects of the trial that are relevant to making the decision to participate, and must have sufficient time and opportunity to ask any questions.

Any change to the content of the ICF must be approved by the IRB prior to the form being used.

If new information becomes available that may be relevant to the subject's willingness to continue participation in the trial, this will be communicated to him/her in a timely manner. Such information will be provided via a revised ICF or an addendum to the original ICF.

Informed consent forms will be photocopied. The original will be kept by the Investigator, and the copy will be kept by the subject.

Documentation of the consent process will be recorded in the source documents.

7.2 Medical History

Prior to enrollment, subjects will be assessed for pre-existing conditions and illnesses, both past and ongoing. This assessment will include a physical examination that will include, but not be limited to, the following body systems: neurological, heart, head, respiratory, and abdomen. Any pre-existing conditions and illnesses will be documented in the source document. A signed medical records release will be requested. Significant medical history (reported as diagnosis) including conditions for which the subject is or has been followed by a physician or conditions that could resume during the course of the study or lead to an SAE or to a repetitive outpatient care will be collected in the electronic case report form (eCRF).

Dates, medications, and body systems will be recorded; the information collected will not be coded.

For each condition, the data collected will be limited to:

- Diagnosis with dates (this is preferable to reporting signs and symptoms).
- Presence or absence of the condition at enrollment.

Vital signs will include temperature, blood pressure, and pulse taken while the subject is in a resting position.

At subsequent visits, a history-driven (targeted) physical examination will focus on areas of complaint by the subject at the discretion of the physician. This may include, but not be limited to, the following body systems: neurological, heart, head, respiratory, and abdomen. The necessity of this physical examination is determined through conversation between the study staff and the subject about the subject's current medical status.

7.3 Screening

Potential subjects will be recruited from the Syracuse, NY area and scheduled for a screening visit. Once a subject signs the informed consent document (see below), they will be assigned a screening number beginning with XXX, XXX+1, etc. When a subject has completed screening and is eligible for participation and they are willing to continue, they will be scheduled for a Day 0 visit. Screen failures will not be entered into the data management system for the study.

Screening Visit (Day -60 to -1)

- Obtain and document consent from potential subject.
- Obtain signed medical records release.
- Collect medical history.
- Assess inclusion and exclusion criteria.
- Perform physical examination, including collection of vital signs and temperature.
- Concomitant medications will be recorded.
- Collect demographics.
- Perform urine pregnancy test.
- Schedule subject for Day 0 visit, if eligible.

7.4 Day 0 Visit - ALL SUBJECTS

- Review consent with subject.
- Review inclusion/exclusion criteria to confirm eligibility.
- Review medical history and note any changes since screening.
- Conduct targeted physical exam, including collection of vital signs and temperature.

- Review concomitant medications.
- Perform urine pregnancy test

  Randomize subject. Schedule will vary depending on group assignment. See Section 7.5 (Groups 1, 2, 3) and 7.6 (Group 4) for detailed schedule.

7.5 Group 1 (Chloroquine), Group 2 (Malarone), Group 3 (Doxycycline)

Day 0

- Collect blood for Research Assays
- Confirm group assignment
- Group 1: Collect urine specimen
- Group 1: Administer chloroquine 500 mg po **OR**

  Group 2: Administer malarone 250/100 mg po and dispense 41 days with instruction **OR**

  Group 3: Administer doxycycline 100 mg po and dispense 41 days with instruction
- Observe subject for 15 minutes and record any adverse reaction(s).

Day 7 (+/- 1 day)

- Conduct targeted physical exam, including collection of vital signs and temperature.
- Review concomitant medications.
- Record any adverse reaction(s).
- Group 1: Collect urine specimen
- Group 1: Confirm group assignment.
- Group 1: Administer chloroquine 500 mg po.
- Group 1: Observe subject for 15 minutes and record any adverse reaction(s).
- Groups 2 and 3: Review drug accountability.

Day 14 (+/- 1 day) Vaccination #1

- Review contraindications to confirm eligibility.
- Perform urine pregnancy test
- Review medical history and note any changes.
- Conduct targeted physical exam, including collection of vital signs and temperature
- Review concomitant medications.
- Record any adverse reaction(s).
- Collect blood for Research Assays (must be completed prior to vaccination)
- Confirm group assignment
- Group 1: Collect urine specimen
- Group 1: Administer chloroquine 500 mg po **OR**

  Groups 2 and 3: Review drug accountability
- Inject RabAvert® vaccine 1.0 mL IM in deltoid.
- Observe subject for 30 minutes and record any adverse reaction(s).

Day 17 (+/- 1 day) Vaccination #2

- Review contraindications to confirm eligibility.
- Perform urine pregnancy test
- Review medical history and note any changes.
- Conduct targeted physical exam, including collection of vital signs and temperature
- Review concomitant medications.
- Record any adverse reaction(s).
- Collect blood for Research Assays (must be completed prior to vaccination)
- Confirm group assignment
- Inject RabAvert® vaccine 1.0 mL IM in deltoid.
- Observe subject for 30 minutes and record any adverse reaction(s).

Day 21 (+/- 1 day) Vaccination #3

- Review contraindications to confirm eligibility.
- Perform urine pregnancy test
- Review medical history and note any changes.
- Conduct targeted physical exam, including collection of vital signs and temperature
- Review concomitant medications.
- Record any adverse reaction(s).
- Collect blood for Research Assays (must be completed prior to vaccination)
- Confirm group assignment
- Group 1: Collect urine specimen
- Group 1: Administer chloroquine 500 mg po **OR**

  Groups 2 and 3: Review drug accountability.
- Inject RabAvert® vaccine 1.0 mL IM in deltoid.
- Observe subject for 30 minutes and record any adverse reaction(s).

Day 28 (+/- 2 days) Vaccination #4

- Review contraindications to confirm eligibility.
- Perform urine pregnancy test
- Review medical history and note any changes.
- Conduct targeted physical exam, including collection of vital signs and temperature
- Review concomitant medications.
- Record any adverse reaction(s).
- Collect blood for Research Assays (must be completed prior to vaccination)
- Confirm group assignment
- Group 1: Collect urine specimen

- Group 1: Administer chloroquine 500 mg po **OR**

  Groups 2 and 3: Review drug accountability.
- Inject RabAvert® vaccine 1.0 mL IM in deltoid.
- Observe subject for 30 minutes and record any adverse reaction(s).

Day 31 (+/- 2 days)

- Conduct targeted physical exam, including collection of vital signs and temperature
- Review concomitant medications.
- Collect blood for Research Assays
- Record any adverse reaction(s).

Day 35 (+/- 2 days)

- Conduct targeted physical exam, including collection of vital signs and temperature
- Review concomitant medications.
- Record any adverse reaction(s).
- Group 1: Confirm group assignment.
- Group 1: Collect urine specimen
- Group 1: Administer chloroquine 500 mg po.
- Group 1: Observe subject for 15 minutes and record any adverse reaction(s).
- Groups 2 and 3: Review drug accountability.

Day 42 (+/- 3 days)

- Conduct targeted physical exam, including collection of vital signs and temperature
- Review concomitant medications.
- Collect blood for Research Assays
- Record any adverse reaction(s).
- Group 1: Collect urine specimen
- Groups 2 and 3: Perform final drug accountability and collect any unused drug.

Day 56 (+/- 7 days)

- Conduct targeted physical exam, including collection of vital signs and temperature
- Review concomitant medications.
- Collect blood for Research Assays
- Group 1: Collect urine specimen
- Record any adverse reaction(s).

7.6 Group 4 (Controls)

Day 0 Vaccination #1

- Collect blood for Research Assays (must be completed prior to vaccination)
- Confirm group assignment
- Inject RabAvert® vaccine 1.0 mL IM in deltoid.
- Observe subject for 30 minutes and record any adverse reaction(s).

Day 3 (+/- 1 day) Vaccination #2

- Review contraindications to confirm eligibility.
- Perform urine pregnancy test
- Review medical history and note any changes.

- Conduct targeted physical exam, including collection of vital signs and temperature
- Review concomitant medications.
- Record any adverse reaction(s).
- Collect blood for Research Assays (must be completed prior to vaccination)
- Confirm group assignment
- Inject RabAvert® vaccine 1.0 mL IM in deltoid.
- Observe subject for 30 minutes and record any adverse reaction(s).

Day 7 (+/- 1 day) Vaccination #3

- Review contraindications to confirm eligibility.
- Perform urine pregnancy test
- Review medical history and note any changes.
- Conduct targeted physical exam, including collection of vital signs and temperature
- Review concomitant medications.
- Record any adverse reaction(s).
- Collect blood for Research Assays (must be completed prior to vaccination)
- Confirm group assignment
- Inject RabAvert® vaccine 1.0 mL IM in deltoid.
- Observe subject for 30 minutes and record any adverse reaction(s).

Day 14 (+/- 1 day) Vaccination #4

- Review contraindications to confirm eligibility.
- Perform urine pregnancy test
- Review medical history and note any changes.
- Conduct targeted physical exam, including collection of vital signs and temperature
- Review concomitant medications.
- Record any adverse reaction(s).

- Collect blood for Research Assays (must be completed prior to vaccination)
- Confirm group assignment
- Inject RabAvert® vaccine 1.0 mL IM in deltoid.
- Observe subject for 30 minutes and record any adverse reaction(s).

Day 17 (+/- 2 days)

- Conduct targeted physical exam, including collection of vital signs and temperature
- Review concomitant medications.
- Collect blood for Research Assays
- Record any adverse reaction(s).

Day 28 (+/- 3 days)

- Conduct targeted physical exam, including collection of vital signs and temperature
- Review concomitant medications.
- Collect blood for Research Assays
- Record any adverse reaction(s).

Day 42 (+/- 7 days)

- Conduct targeted physical exam, including collection of vital signs and temperature
- Review concomitant medications.
- Collect blood for Research Assays
- Record any adverse reaction(s).

8 SAMPLE COLLECTION

Blood samples will be collected for Research Laboratory Assays according to the Blood Draw Schedule in Appendix A. Immediately prior to the blood draw; the staff member performing the procedure will remove the collection tubes from the supplied kit and verify the subject's ID number on the labels. Blood will be taken prior to vaccination(s).

In the event of an AE or SAE, additional biological samples (e.g., additional blood, body fluids, tissue samples) may be collected.

8.1 Sample Preparation

Blood samples will be retrieved and processed according to Center for Global Health and Translational Science (CGHATS) Standard Operating Procedures (SOP) or Study Specific Procedures (SSP). Briefly, serum samples will be maintained at room temperature for the allotted time, centrifuged, aliquoted and frozen at -80°C for long term storage. Peripheral Blood Mononuclear Cell (PBMC) samples will be centrifuged, plasma will be removed and retained, cells will be washed, resuspended in freezing media, aliquoted and frozen in liquid nitrogen vapor phase for long term storage.

8.2 Laboratory Assays

8.2.1 Rapid Fluorescent Foci Inhibition Test (RFFIT)

RFFIT is a serum neutralization (inhibition) test, which means it measures the ability of rabies specific antibodies to neutralize rabies virus and prevent the virus from infecting cells. These antibodies are called rabies virus neutralizing antibodies (RVNA).

The World Health Organization (WHO) guideline of 0.5 International Units per milliliter (IU/mL) is used as an adequate response to rabies vaccination, as well as the ACIP level of "complete neutralization at a serum dilution of 1:5 in the RFFIT test" as evidence of adequate response in a person.

RFFIT analysis will be performed on samples taken from Day 0, prior to vaccination 3, prior to vaccination 4, 14 and 28 days post 4th vaccination.

8.2.2 Proteomics

Proteomics is the analysis of the expression, localization, function, and interactions of a set of proteins expressed by the genetic material of an organism under a given set of environmental conditions, using iTRAQ or equivalent method. This technique offers a novel method to evaluate clinical samples from experimental inoculation for identification of novel biomarkers and correlates of immunogenicity. Proteomics may help elucidate the mechanisms by which antimalarials alter the immune response to rabies vaccination. Proteomics analysis can be performed on either serum or plasma samples. Proteomics is an exploratory assay and may or may not be done based on

whether a statistically significant difference in GMT is detected between study groups and on data collected from other ongoing studies.

8.2.3 Cellular Assays

The cellular response to rabies vaccination is an exploratory assay and may or may not be done based on whether or not a difference in GMT is detected between study groups and data collected from other ongoing studies.

8.2.4 Urine Analysis

Urine will be analyzed to determine if chloroquine is excreted in part, bound to saposin B. Saposin B may play a role in mitigating chloroquine toxicity and/or may be a source of toxicity associated with long term chloroquine use.

8.2.5 Sample Storage and Shipment

Samples will be stored in temperature controlled units, -80°C ± 10°C for serum and plasma samples, vapor phase liquid nitrogen for PBMCs. These units are monitored 24 hours per day and documentation of the temperature will be maintained throughout the trial. Any temperature deviations resulting in an alarm condition will be noted on the shipping manifest.

At mutually agreed upon times, samples will be shipped for testing according to CGHATS SOP. Sera will be shipped frozen, using dry ice to maintain a frozen state. PBMCs will be shipped using dry shippers. Shipments must be compliant with the International Air Transport Association (IATA) 602 regulations and temperature will be monitored while in transit.

9 ASSESSMENT OF SAFETY

Although using only licensed products for this study, subjects will be kept under observation for 15 minutes after the first dose of antimalarial and 30 minutes after each rabies vaccination to ensure their safety. Any AE that occurs during this period will be noted on the source document and identified as an immediate event / reaction; and will additionally be recorded in the eCRF.

9.1 Specification of Safety Parameters

9.1.1 Unanticipated Problems

Federal regulations require that unanticipated problems involving risks to subjects or others be promptly reported to the IRB. These events encompass a broader category of events than SAEs and may include issues such as problems with loss of control of subject data or the investigational product, adverse psychological reactions, or breach of confidentiality. Risks to others (eg, program personnel) must also be reported.

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

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

The IRB and the HRPO will evaluate the PI’s and research monitor’s reports to determine whether a given incident, experience or outcome constitutes an unanticipated problem involving risk to subjects or others and ensure upward reporting of the unanticipated problems involving risk to subjects or others to the appropriate regulatory offices.

9.1.2 Adverse Events

An adverse event is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject’s

participation in the research, whether or not considered related to the subject's participation in the research.

9.1.3 Serious Adverse Events

A serious adverse event (SAE) is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

9.2 Time Period and Frequency for Event Assessment and Follow-Up

Unanticipated problems will be recorded in the data collection system throughout the study.

The PI will record all reportable events with start dates occurring any time after informed consent until study completion. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

Any SAE that occurs after a subject has completed the study but that is likely to be related to the product or to the experiment must also be reported as soon as possible.

9.3 Characteristics of an Adverse Event

9.3.1 Relationship to Study Intervention

To assess relationship of an event to study intervention, the following guidelines are used:

1. Related (Possible, Probable, Definite)
    a. The event is known to occur with the study intervention.
    b. There is a temporal relationship between the intervention and event onset.

c. The event abates when the intervention is discontinued.

d. The event reappears upon a re-challenge with the intervention.

2. Not Related (Unlikely, Not Related)

a. There is no temporal relationship between the intervention and event onset.

b. An alternate etiology has been established.

9.3.2 Severity of Event

The following scale will be used to grade adverse events:

1. Mild: does not interfere with routine activities; minimal level of discomfort
2. Moderate: interference with routine activities; moderate level of discomfort
3. Severe: unable to perform routine activities; significant level of discomfort

9.4 Reporting Procedures

The PI will report all AEs to the SUNY IRB and the USAMRMC ORP HRPO in the appropriate safety, annual, and/or final reports.

9.4.1 Unanticipated Problem Reporting to IRB

Incidents or events that meet the requirements for notifying the SUNY IRB of unanticipated problems will be reported to the SUNY IRB using the following timeline:

- Unanticipated problems that are serious adverse events will be reported to the IRB within 1 week of the investigator becoming aware of the event.
- Any other unanticipated problem will be reported to the IRB within 2 weeks of the investigator becoming aware of the problem.

9.4.2 Serious Adverse Event Reporting to HRPO

Any Serious Adverse Event will be submitted to the USAMRMC ORP HRPO and the research monitor.

Initial reports of SAEs should be sent by the PI within 72 hours of the Investigator becoming aware of an SAE. Any new relevant information concerning the SAE (e.g., outcome, precise description of medical history, results of the investigation) should be sent as soon as possible. Copies of documents (e.g., medical records, discharge summary, autopsy) may be requested.

Notification of an SAE to the Research Monitor for the study, email: *fazilit@upstate.edu* and the Human Research Protection Office (HRPO) email: *usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil* Human Research Protection Office (HRPO), U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RPH, 504 Scott Street, Frederick, MD 21702-5012 will be done in parallel.

All SAEs will be followed until resolution or stabilization.

9.4.3 Reporting of Pregnancy

If the Investigator becomes aware of pregnancy of study participant, paper pregnancy reporting form should be completed and sent within 72 hours. The investigator will notify the research monitor, email: *fazilit@upstate.edu* and the Human Research Protection Office (HRPO) email: *usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil*.

Study staff must then maintain contact with the subject to obtain information about the outcome, i.e., details about the delivery and the newborn, or about pregnancy termination. Pregnancy itself is not considered an AE, but any complications during pregnancy are to be considered as AEs, and in some cases could be considered SAEs. Spontaneous abortions, fetal death, stillbirth, and congenital anomalies reported in the baby are always considered as SAEs, and the information should be provided using an SAE form.

10 STUDY OVERSIGHT

10.1 Research Monitor

In addition to the PI's responsibility for oversight, study oversight will be under the direction of a Research Monitor. The research monitor is independent of the study and will be available in real time to review and recommend appropriate action regarding adverse events and other safety issues.

Per requirements of Department of Defense Instructions 3216.02, research funded with Department of Defense funds requires a research monitor to be appointed. Dr. Tasaduq Fazili will be the research monitor for this trial. His duties will include reviewing and providing and unbiased assessment and written report of all: SAEs, Subject deaths, Unexpected AEs, Protocol deviations that are related to an adverse event impacting the subject, Annual reports and Unanticipated problems involving risks to subject and others involved in the trial. The research monitor may observe recruitment and enrollment procedures and the consent process for individuals, groups or units, review monitoring plans and data collection.

The research monitor may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research. He shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report. He shall have the responsibility to promptly report his observations and findings to the IRB or other designated official and the Human Research Protection Office.

11 CLINICAL SITE MONITORING

Clinical site monitoring is conducted to ensure that the rights of human subjects are protected, that the study is implemented in accordance with the protocol and/or other operating procedures, and that the quality and integrity of study data and data collection methods are maintained. The monitor will evaluate study processes and documentation based on International Conference on Harmonisation (ICH), E6: Good Clinical Practice guidelines (GCP).

Before the start of the trial (i.e., before the inclusion of the first subject), the Investigators and the Monitoring representative will meet at the site-initiation visit to discuss the trial protocol and the detailed trial procedures. Emphasis will be placed on inclusion and exclusion criteria, visit timing, informed consent procedures, eCRF completion, and the handling of samples and products.

The monitoring staff will ensure and document that all material to be used during the trial has been received at the site; and that the study investigator team and local monitoring staff have been properly informed about the trial, GCP and regulatory requirements.

After the start of the trial, the monitoring staff or a representative will be in regular contact with the investigational team through telephone calls and regular follow-up visits. The Investigator or delegate must be available for these visits, and must allow the monitoring staff direct access to subject medical files and eCRFs. During these visits, the monitoring staff will:

- Evaluate the quality of the trial progress (adherence to protocol and any study-specific guidelines, quality of data collection and document completion, signature of consent forms, occurrence of SAEs, sample and product management, cold-chain monitoring, archiving, and direct observation of study procedures during the trial).
- Source-verify completed eCRFs and any corresponding answered queries.
- Determine the number of complete or ongoing issues identified at monitoring visits (e.g., protocol violations, SAEs). Any identified problems will be discussed with the Investigator, and corrective or preventive actions will be determined, as appropriate.
- Review that all protocol procedures have been completed and the data have been entered into the eCRF. All data-related queries must be completed prior to database lock.

At the end of the trial, a close-out visit will be performed to ensure that:

- CGHATS has all the documents necessary for archiving.
- All samples have been shipped to the appropriate laboratories.
- All unused products have been either destroyed or accounted for.

12 STATISTICAL CONSIDERATIONS

12.1 Study Hypotheses

This will be a descriptive study to assess immunogenicity of a licensed rabies vaccine using test dosage schedules in the presence of malaria chemoprophylaxis. No formal statistical hypothesis tests will be conducted. Descriptive analyses will be based on the per-protocol analysis sets.

Exploratory statistical analyses of final data may be conducted, if indicated by the descriptive results. Parametric, non-parametric and resampling (bootstrapping) methods for statistical inference may be used in exploratory analyses, based on data compliance with assumptions of methods. P -values ≤ 0.05 will be considered significant and p-values ≤ 0.10 will be considered a trend. Confidence intervals will be constructed at α=0.05 and α=0.10. When necessary, p-value corrections for multiple comparisons will be applied.

Descriptive and any inferential analyses will be carried out using SAS Version 9.2 (or higher), which is licensed and supplied by SAS Institute, Cary, NC, USA.

12.2 Sample Size Considerations

The planned sample size is 100 subjects, who will be randomly allocated (1:1:1:1) into three treatment groups and one control group. The sample size was chosen empirically, based on sample sizes of other exploratory studies of this general type. This exploratory study is not powered to formally test equivalency or non-inferiority in immunogenicity among groups according to malaria prophylaxis. Subjects who withdraw prior to first vaccination will be replaced by the next enrolled subject who will be assigned to the same group as the withdrawn subject. Replacement subjects will not be randomized.

12.2.1 Safety Review

A descriptive summary of adverse events that were reported throughout the trial will be produced.

12.3 Final Analysis Plan

Primary Objective Analysis

Descriptive analyses for the primary endpoints will be based on per-protocol analysis data sets using the RFFIT data from blood samples taken 14 days post completion of all four vaccine doses. Separate descriptive analyses will be conducted for each per-protocol data set and group.

Secondary Objective Analysis

Descriptive analyses for the secondary endpoints will be based on per-protocol analysis data sets using the RFFIT data from blood samples taken prior to third and fourth dose of rabies vaccine and 28 days post fourth vaccine dose.

Per Protocol Analysis Set

The Per-Protocol (PP) analysis set will include all subjects who had no protocol deviations at the time that the data was collected. Two PP analysis sets will be produced for the primary objective that will contain separate data from blood drawn on day 0 and 14 days post 4^{th} vaccination. Descriptive analysis of the secondary endpoint will require three additional per protocol analysis sets for blood drawn prior to 3^{rd} and prior to 4^{th} vaccination and on day 28 post 4^{th} vaccination.

Subjects will be excluded from the PPAS for the following reasons:

1. Subject did not meet all protocol-specified inclusion/exclusion criteria or experienced a definitive contraindication

2. Administration of vaccine was not done per protocol

3. Subject did not receive vaccine in the proper time window as defined in the study procedures

4. Subject did not provide a post-dose serology sample in the proper time window as defined in the study procedures*

5. For the malarone and doxycycline groups, subject missed the three or more of the daily dose of antimalarial in any one week.

6. For the chloroquine group, subject missed their weekly dose by three or more days

*Subjects whose serology sample is outside the defined window for the secondary endpoint will still be included in the primary endpoint analysis.

For the computation of GMTs, a titer reported as < LLOQ will be converted to a value of 0.5 LLOQ.

For calculating fold-rise and titer ratio (GMTR), < LLOQ will be converted to 0.5 LLOQ for a numerator and < LLOQ will be converted to LLOQ for a denominator

Any titer reported as > ULOQ (upper limit of quantization) will be converted to ULOQ.

Missing data will not be imputed.

Potential outliers will be identified using histograms and modified box plots or normal probability plots, when appropriate. Choice of subsequent tests for outliers (e.g. Grubbs, Tietjen-Moore and ESD) will depend on the number and nature of suspected outliers and may require data transformation. Bootstrapped estimates of standard errors may also be reported. Sensitivity analysis of effect of outliers on descriptive statistics will be conducted, and if necessary descriptive statistics that include and exclude outliers will be reported

13 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Source documents are original documents or certified copies, and include, but are not limited to medical and hospital records, screening logs, informed consent forms, telephone contact logs, and worksheets. The purpose of trial source documents is to document the existence of subjects and to substantiate the integrity of the trial data collected. Investigators must maintain source documents so that they are accurate, complete, legible, and up to date.

The subject screening and enrollment log should list all individuals contacted by the Investigators to participate in the trial, regardless of the outcome.

Source documents will be stored in a secure location with access restricted to study personnel for the protection of confidentiality of subjects.

Study staff will permit authorized representatives of Upstate Medical University and regulatory agencies to examine (and when required by applicable law, to copy) research records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress and data validity.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Quality Control in Data

Accuracy of data in the database is reviewed on numerous levels. Every attempt is made by the CRC to review source documentation prior to the subject leaving the site. Data is reviewed during data entry, usually completed within 24 hours of the subject visit. Source document to database entry is monitored 100% by the clinical monitor. Any discrepancies between the source and database will be corrected by the CRC.

Product accountability is the responsibility of the principal investigator or designee. Documentation pertaining to product accountability are monitored by the clinical monitor and SUNY-UMU Pharmacy.

14.2 Quality Assurance in Clinical Study

During review of a clinical protocol, the SUNY IRB ensures that staff, who will be part of the clinical study, are properly trained to perform the tasks assigned. Additionally, training records are reviewed by the clinical monitor.

The SUNY IRB works very closely with the SUNY Quality Assessment and Improvement Programs (QAIP) which is a post (IRB) approval monitoring program aimed at providing subjects with an extra level of protection by reviewing the conduct of the study in real time. In the event a quality issue arises, the QAIP office will make recommendations for a corrective action and it is the responsibility of the principal investigator to ensure the corrective action has been implemented.

15 PROTECTION OF HUMAN SUBJECTS

15.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in the Declaration of Helsinki (2008) as far as adopted by the concerned regulatory authorities as well as in accordance with 45 CFR Part 46 and/or the ICH E6. The investigator will ensure that this study is conducted in full conformity with the applicable national and local requirements regarding ethical committee review, informed consent, and other statutes or regulations regarding the protection of the rights and welfare of human subjects participating in biomedical research.

15.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study.

Copies of these approvals, along with information on the type, version number, and date of document, and the date of approval, must be forwarded by the Investigator HRPO.

15.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Extensive discussion of risks and possible benefits of study participation will be provided to subjects and their families, if applicable. A consent form describing in detail the study procedures and risks will be given to the subject. Consent forms will be IRB-approved, and the subject is required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the subject and answer any questions that may arise. The subject will sign the informed consent document prior to any study-related assessments or procedures. Subjects will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study.

The consent process will be documented in the clinical or research record.

15.4 Subject Confidentiality

Subject confidentiality is strictly held in trust by the investigators, study staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples in addition to any study information relating to subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized party without prior approval of the Principal Investigator.

The study monitor may inspect all study documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the study subjects. The clinical study site will permit access to such records.

The IRBs, and regulatory agencies, including the FDA, require direct access to all study records, and will treat these documents in a confidential manner.

15.5 Future Use of Stored Specimens and Other Identifiable Data

Any unused part of the research samples will be securely stored at WRAIR or Upstate Medical University for at least 5 years.

Subjects will be asked to indicate on the ICF whether they will permit the future use of any unused samples for other tests. If they refuse permission, the samples will not be used for any testing other than that directly related to this study. If they agree to this use, they will not be paid for giving permission. (Anonymity of samples will be ensured.) The aim of any possible future research is unknown today, and may not be related to this particular study. It may be to improve the knowledge of vaccines or infectious diseases, or to improve laboratory methods. Genetic tests will never be performed on these samples without individual informed consent.

16 DATA HANDLING AND RECORD KEEPING

The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

At specified intervals, the Investigator or an authorized designee will interview the subjects to collect information, and will attempt to clarify anything that is incomplete or unclear. All clinical trial information gathered by the study site will be reported electronically by the Investigator or authorized designee using a web-based eCRF held at Frontier Science in Amherst, NY. The eCRF has been designed specifically for this trial using a validated Electronic Records / Electronic Signature-compliant platform (21CFR Part 11).

16.1 Data Management Responsibilities

Data collection and accurate documentation are the responsibility of the study staff under the supervision of the investigator. All source documents and laboratory reports must be reviewed by the study team and data entry staff, who will ensure that they are accurate and complete. Unanticipated problems and adverse events must be reviewed by the investigator or designee.

Data monitoring at the sites and quality control in the form of computerized logic and / or consistency checks will be systematically applied in order to detect errors or omissions. Any questions pertaining to the reported clinical data will be submitted to the Investigator for resolution using the Electronic Data Capture (EDC) system. Each step of this process will be monitored through the implementation of individual passwords to maintain appropriate database access and to ensure database integrity.

The Investigator is responsible for the timeliness, completeness, and accuracy of the information in the eCRFs; must provide explanations for all missing information; and must sign the eCRF using an e-signature.

16.2 Data Capture Methods

Clinical source data will be captured on paper and entered into electronic database by study staff.

Upon completion of training, each user requiring access to the EDC system will be issued a unique username and password. In the event of a change in trial personnel, each newly assigned individual will receive a unique username and password; the username and password of a previous user may not be reissued.

Immunogenicity data will be performed at the laboratory level following the laboratory's procedures. Information from the laboratory will be checked for consistency before integration into the clinical database.

After integration of all corrections in the complete set of data, the database will be released for statistical analysis.

16.3 Study Records Retention

The Investigator will retain all source documents, consent forms, for at least 2 years after the investigation is discontinued.

The Investigator will retain all documentation pertaining to the trial. The protocol, documentation, approvals, and all other documents related to the trial, including certificates attesting that satisfactory audit and inspection procedures have been carried out will be kept by Upstate in the Investigator's File. Data on AEs are included in the Investigator's file. Archived data may be held on microfiche or electronic records, provided that a back-up exists and that a hard copy can be obtained if required. All data and documents will be made available if requested by relevant authorities.

16.4 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice, or Manual of Procedures requirements. The noncompliance may be on the part of the subject, the investigator, or study staff. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

These practices are consistent with investigator and sponsor obligations in ICH E6:

- Compliance with Protocol, Sections 4.5.1, 4.5.2, 4.5.3, and 4.5.4.
- Quality Assurance and Quality Control, Section 5.1.1
- Noncompliance, Sections 5.20.1 and 5.20.2.

All deviations from the protocol must be addressed in study subject source documents and reported to the IRB according to their requirements.

17 LITERATURE REFERENCES

(1) World Health Organization (WHO): Rabies Fact Sheet No 99. Available at *http://www.who.int/mediacentre/factsheets/fs099/en/* ; accessed 25 July 2015.

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(3) Pappaioanou M, Fishbein DB, Dreesen DW, Schwartz IK, Campbell GH, Sumner JW, Patchen LC, Brown WJ. Antibody response to preexposure human diploid-cell rabies vaccine given concurrently with chloroquine. N Engl J Med. 1986 Jan 30;314(5):280-4.

(4) Taylor DN, Wasi C, Bernard K. Chloroquine prophylaxis associated with a poor antibody response to human diploid cell rabies vaccine. Lancet. 1984 Jun 23;1(8391):1405.

(5) Rupprecht CE, Briggs D, Brown CM, et al, Use of a Reduced (4-Dose) Vaccine Schedule for Postexposure Prophylaxis to Prevent Human Rabies: Recommendations of the Advisory Committee on Immunization Practices, MMWR March 19, 2010 / 59(RR02);1-9.

(6) Moe CD, Keiser PB, Should U.S. troops routinely get rabies pre-exposure prophylaxis? Mil Med. 2014 Jul;179(7):702-3.

APPENDIX A: SCHEDULE OF EVENTS

GROUP 1 (Chloroquine), GROUP 2 (Malarone), GROUP 3 (Doxycycline)

Study Day	-60 to 0	0	7	14	17	21	28	31	35	42	56
Visit Type	SCR	BL		BL VAC	BL VAC	BL VAC	BL VAC	BL		BL	BL
Time Windows (days)	-60 to 0	0	+/- 1	+/- 1	+/- 1	+/- 1	+/- 2	+/- 2	+/- 2	+/- 3	+/- 7
Informed Consent IC Review	X	X									
Inclusion/Exclusion Criteria	X	X									
Medical Records Release	X										
Contraindications				X	X	X	X				
Medical History	X	X		X	X	X	X				
Physical Exam	X										
Targeted Physical Exam [a]		X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X
Urine Pregnancy Test [b]	X	X		X	X	X	X				
Concomitant Medication	X	X	X	X	X	X	X	X	X	X	X
Demography	X										
Randomization		X									
Confirm Group Assignment [c]			X	X	X	X	X		X		
Blood Draw for Research		X		X	X	X	X	X		X	X
Collect Urine Group 1		X	X	X		X[e]	X[e]		X[e]	X[e]	X[e]
Dispense Medication Group 1		X	X	X		X	X		X		
Dispense Medication Groups 2 and 3		X									
Review Medication Groups 2 and 3			X	X		X	X		X		

Vaccination				X	X	X	X				
Study Day	-60 to 0	0	7	14	17	21	28	31	35	42	56
Final Drug Accountability Groups 2 and 3										X	
AE/SAE		X	X	X	X	X	X	X	X	X	X
15 min post dose observation [d]		X	X						X		
30 min post vaccine observation				X	X	X	X				

a – At Investigator's discretion
b – Female subjects only
c – Confirm group assignment on Day 7 & Day 35 applies to Group 1 only
d – 15 minute observation on Day 7 & Day 35 applies to Group 1 only

e – Urine samples may not be collected at later dates pending results from early samples

APPENDIX B: SCHEDULE OF EVENTS

GROUP 4 (Control)

Study Day	-60 to 0	0	3	7	14	17	28	42
Visit Type	Screening	BL VAC	BL VAC	BL VAC	BL VAC	BL	BL	BL
Time Windows (days)	-60 to 0	0	+/- 1	+/- 1	+/- 1	+/- 2	+/- 3	+/- 7
Informed Consent/IC Review	X	X						
Medical Records Release	X							
Inclusion/Exclusion Criteria	X							
Contraindications		X	X	X	X			
Medical History	X	X	X	X	X			
Physical Exam	X							
Targeted Physical Exam [a]		X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X
Urine Pregnancy Test [b]	X	X	X	X	X			
Concomitant Medication	X	X	X	X	X	X	X	X
Demography	X							
Randomization		X						
Blood Draw for Research		X	X	X	X	X	X	X
Confirm Group Assignment		X	X	X	X			
Vaccination		X	X	X	X			
AE/SAE		X	X	X	X	X	X	X
30 minute post vaccine observation		X	X	X	X			

a – At Investigator's discretion
b – Female subjects only

APPENDIX C: Blood Draw Schedule

GROUP 1 (Chloroquine), GROUP 2 (Malarone), GROUP 3 (Doxycycline)

Study Day	0	7	14	17	21	28	31	35	42	56
Serum (8.5 mL)	1		1	1	1	1	1		1	1
PBMC (8.0 mL)			5		3	3			5	5
Daily BV (mL)	8.5	0	48.5	8.5	32.5	32.5	8.5	0	48.5	48.5
CBV (Total)	8.5	8.5	57	65.5	98	130.5	139	139	187.5	236
Total (56 Days)										236

GROUP 4 (Control)

Study Day	0	3	7	14	17	28	42
Serum (8.5 mL)	1	1	1	1	1	1	1
PBMC (8.0)	5		3	3		5	5
Daily BV (mL)	48.5	8.5	32.5	32.5	8.5	48.5	48.5
CBV (Total)	48.5	57	89.5	122	130.5	179	227.5
Total (56 Days)							227.5