

Title: Immunogenicity of a Two vs Three dose, Intradermal (ID) vs Intramuscular (IM) Administration of a Licensed Rabies Vaccine for Pre-Exposure Vaccination

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Immunogenicity of a Two vs Three dose, Intradermal (ID) vs Intramuscular (IM) Administration of a Licensed Rabies Vaccine for Pre-Exposure Vaccination

Randomized, open-label, single center trial in adults aged 18 to 60 years in the US

Clinical Trial Protocol Version 6.0 dated 20 MAY 2016

Funder's Representative:

LTC(P) Paul Keiser
Walter Reed Army Institute of Research
503 Robert Grant Avenue
Silver spring, MD 20910

Principal Investigator:

Mark E Polhemus
Upstate Medical University
725 Irving St. Suite 311
Syracuse, NY 13210

Licensed Products:

The RabAvert® Rabies Vaccine

Form /Route:

Intradermal and Intramuscular

Indication For This Study:

Comparison of Dose and Schedule

Research Monitor:

Tasaduq Fazili, MD
Upstate Medical University
725 Irving Ave., Suite 311
Syracuse, NY 13210

Funder's Clinical Development

Director:

Lou Jasper
USAMMDA
1430 Veteran's Drive
Ft Detrick, MD 21702
Tel: (301) 619-4951
Fax: (301) 619-2304

Study Site/PI Location:

Center for Global Health and Translational Science
Upstate Medical University
Institute for Human Performance
505 Irving Avenue
Syracuse, NY 13210

Table of Contents

Synopsis.....	4
List of Terms and Abbreviations.....	23
1 Introduction.....	25
1.1 Background of Rabies.....	25
1.2 Background of the Licensed Product.....	26
1.3 Potential Benefits to Subjects.....	27
1.4 Potential Risks to Subjects.....	27
1.5 Rationale.....	28
2 Objectives.....	29
2.1 Primary Objectives.....	29
2.2 Secondary Objectives.....	29
2.3 Exploratory Objectives.....	30
3 Investigators and Trial Organization.....	30
4 Independent Ethics Committee/ Institutional Review Board.....	30
5 Trial Design.....	30
5.1 Justification of the Trial Design.....	31
5.2 Trial Plan.....	32
5.3 Visit Procedures.....	32
5.4 Planned Trial Calendar.....	45
6 Enrollment and Retention of Trial Population.....	45
6.1 Recruitment Procedures.....	45
6.2 Informed Consent Procedures.....	45
6.3 Screening Criteria.....	45
6.4 Inclusion Criteria.....	46
6.5 Exclusion Criteria.....	46
6.6 Medical History.....	47
6.7 Contraindications for Subsequent Vaccinations.....	47
6.7.1 Temporary Contraindications.....	47
6.7.2 Definitive Contraindications.....	47
6.8 Other Conditions-for-Withdrawal beyond Definitive Contraindications.....	48
6.9 Lost to Follow-up Procedures.....	49
6.10 Classification of Subjects Who Discontinue the Trial.....	49
6.10.1 Follow-up of Discontinuations.....	49
6.10.2 Follow-up and Reporting of Pregnancies.....	49
6.11 Modification of the Trial and Protocol.....	50
7 Safety Emergency Coverage.....	50
8 Vaccines Administered.....	50
8.1 Identity of Trial Product: Rabies Vaccine.....	51
8.1.1 Composition.....	51
8.1.2 Preparation and Administration.....	52
8.1.3 Dose Selection and Timing.....	52
8.1.4 Labeling and Packaging.....	52
8.1.5 Product Shipment.....	52

8.1.6 Product Storage.....	52
8.1.7 Product Accountability.....	52
8.1.8 Replacement Doses.....	52
8.1.9 Disposal of Unused Products.....	52
9 Blinding and Code-breaking Procedures.....	53
10 Randomization and Allocation Procedures.....	53
11 Treatment Compliance.....	53
12 Concomitant Medication or Other Therapies.....	53
13 Management of Samples.....	55
13.1 Sample Collection.....	55
13.1.1 Blood Samples.....	55
13.1.2 Additional Biological Samples.....	55
13.2 Sample Preparation.....	55
13.3 Sample Storage and Shipment.....	56
13.4 Future Use of Stored Serum Samples for Research.....	56
14 Clinical Supplies.....	56
15 Endpoints and Assessment Methods.....	56
15.1 Primary Endpoints.....	56
15.1.1 Immunogenicity Endpoints.....	56
15.2 Secondary Endpoints.....	57
15.2.1 Immunogenicity Endpoints.....	57
15.3 Assessment Methods.....	57
16 Reporting AEs to IRBs.....	57
17 Reporting of Serious Adverse Events.....	57
Timelines and process for SAE reporting.....	57
Process for SAE documentation.....	58
Timelines and process for pregnancy reporting.....	58
17.1 Follow-up Reporting by the Investigator.....	58
17.2 Reporting of SAEs Occuring After a Subject Has Completed the Study.....	58
17.3 Assessment of Causality.....	58
17.4 Reporting SAEs to Health Authorities and IRBs.....	59
18 Data.....	59
18.1 Data Collection and eCRF Completion.....	59
18.2 Data Management.....	60
18.3 Data Review.....	60
19 Statistical Methods.....	60
19.1 Statistical Methods for Primary Immunogenicity Objectives.....	61
19.2 Statistical Methods for Secondary Immunogenicity Objectives.....	61
19.3 Per-Protocol Analysis Set.....	61
19.4 Full Analysis Set.....	62
19.5 Populations Used in Analysis.....	62
20 Handling of Missing Data and Outliers.....	62
20.1 Immunogenicity.....	62
21 Determination of Sample Size and Power Calculation.....	63
22 Ethical and Legal Issues and Investigator/Sponsor Responsibilities.....	63

22.1 Ethical Conduct of the Trial/Good Clinical Practice.....	63
22.2 Source Data and Source Documents.....	63
22.3 Confidentiality of Data and Access to Subject Records.....	63
22.4 Monitoring, Auditing, and Archiving.....	64
22.4.1 Monitoring.....	64
22.4.2 Audits and Inspections.....	64
22.4.3 Archiving.....	64
23 Stipends for Participation.....	65
24 Reference List.....	65

Synopsis

Licensed Product:	RabAvert® Rabies Vaccine																																														
Title of the Trial:	Immunogenicity of a two vs three dose, intradermal (ID) vs intramuscular (IM) administration of rabies vaccine for pre-exposure vaccination																																														
Trial Centers:	Single site trial conducted at the State University of New York (SUNY) Upstate Medical University Center for Global Health and Translational Science																																														
Planned Trial Period:	February 2014: IRB Submission Planned trial period - FVFS (first visit, first subject) to LCLS (last contact, last subject): October 2014 February 2016 October 2014: Enrollment October 2014: First subject, first vaccination January 2015: Last subject, last vaccination February 2016: Last contact, last subject/Final study visit July 2016: Assays Complete January 2017: Final clinical study report submission																																														
Trial Design and Methodology:	This will be a randomized, open-label, single center study involving 60 healthy adults aged \geq 18 years to \leq 60 years at the time of first vaccination. Table S1: Schedules of vaccine administration and number of subjects by group <table border="1" data-bbox="409 1051 1449 1522"> <thead> <tr> <th rowspan="2">Vaccine Group</th> <th colspan="4">Day</th> <th rowspan="2">Number per Group</th> </tr> <tr> <th>0</th> <th>7</th> <th>21</th> <th>365 (Boost)</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>IM</td> <td>IM</td> <td>IM</td> <td>IM</td> <td>12</td> </tr> <tr> <td>2</td> <td>ID</td> <td>ID</td> <td>ID</td> <td>IM</td> <td>12</td> </tr> <tr> <td>3</td> <td>IM</td> <td>IM</td> <td></td> <td>IM</td> <td>12</td> </tr> <tr> <td>4</td> <td>ID</td> <td>ID</td> <td></td> <td>IM</td> <td>12</td> </tr> <tr> <td>5</td> <td>IM control</td> <td></td> <td></td> <td></td> <td>6</td> </tr> <tr> <td>6</td> <td>ID control</td> <td></td> <td></td> <td></td> <td>6</td> </tr> </tbody> </table> In all groups, subjects will provide blood samples for determination of neutralizing antibody (Ab) titers. See attached Tables of Study Procedures for summary of assays for each visit.	Vaccine Group	Day				Number per Group	0	7	21	365 (Boost)	1	IM	IM	IM	IM	12	2	ID	ID	ID	IM	12	3	IM	IM		IM	12	4	ID	ID		IM	12	5	IM control				6	6	ID control				6
Vaccine Group	Day				Number per Group																																										
	0	7	21	365 (Boost)																																											
1	IM	IM	IM	IM	12																																										
2	ID	ID	ID	IM	12																																										
3	IM	IM		IM	12																																										
4	ID	ID		IM	12																																										
5	IM control				6																																										
6	ID control				6																																										

	<p>Subjects will be randomly assigned to one of the six groups 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.</p> <p>After screening visit, eligible subjects that are randomized will be assigned a unique subject ID. The subject ID will remain unchanged throughout the study and the nurse will confirm the number at every visit. No single subject will receive more than one subject ID. Blood will be drawn at each follow-up visit to be used in research assays for antibody response, and proteomics evaluation. See blood draw table for timing and volumes of blood draws and assays for which they will be used.</p> <p>In all groups additional blood samples may be taken if required to assess AEs, SAEs, or abnormal laboratory findings.</p>
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Primary Objective:	<i>Immunogenicity</i> 1) To describe the percentage of subjects achieving a protective humoral immune response at 12 months across immunization routes at 7 days post boost.
Primary Endpoints:	<i>Immunogenicity</i> 1) Percentage of subjects in each group with titer of IgG antibodies above the protective limits ($>$ or $=$ 0.5 IU/ml) at 12 months, 7 days after final vaccine dose.
Secondary Objectives:	<i>Immunogenicity</i> 1) To describe the percentage of subjects maintaining a protective humoral immune response at 12 months across immunization routes.
Secondary Endpoint:	<i>Immunogenicity</i> 1) Percentage of subjects in each group with titer of IgG antibodies above the protective limits ($>$ or $=$ 0.5 IU/ml) at 12 months, prior to boost.

Exploratory Objectives	<i>Immunogenicity</i> 1) Describe the cell-mediated immune response to rabies vaccination in groups 1-4. 2) Proteomic characterization of the host response before and after each vaccination to identify biomarkers and correlates of immunity
Exploratory Endpoints	<i>Immunogenicity</i> 1) Frequency, kinetics, and/or phenotype of virus immune cell subsets (e.g., B cells, T cells) at baseline and in response to rabies vaccination in groups 1-4. 2) Development of Proteome 3D for complete seroconversion, incomplete seroconversion, and severe reaction. 3) Additional analyses not specified here may be performed in order to further characterize vaccine responses.

Planned Sample Size:	<p>A total of 60 subjects will be vaccinated.</p> <p>There will be four groups (Groups 1, 2, 3, 4, with 12 subjects in each group) and two groups (Groups 5 and 6 with 6 subjects per group.)</p>								
	<p>Screening visit: All screened subjects will review and sign an informed consent form, and undergo a thorough medical history and physical examination to assess for inclusion/exclusion criteria. All females will provide a urine sample to assess for pregnancy. Screening will be conducted within 60 days of vaccination. Screening numbers will be assigned when the subject signs the consent document starting with screening #100, 101, 102, etc. When the subject has completed the screening visit and is eligible and willing to continue participation, they will be randomized according to the randomization process outlined in section 6.4. Screen-fails will not be recorded in the data management system for the study.</p> <p>Vaccinations and Blood Sampling: All subjects will provide a blood sample before the first vaccination for baseline research labs. Results of vaccination day labs will not be reviewed before immunization.</p>								
Schedule of Study Procedures:	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th data-bbox="373 768 491 811" style="text-align: center;">Group</th><th data-bbox="491 768 1519 811" style="text-align: center;">Schedule of Vaccination and Blood Draw</th></tr> </thead> <tbody> <tr> <td data-bbox="373 811 491 1079" style="text-align: center;">1</td><td data-bbox="491 811 1519 1079"> <p>Vaccinations: Rabies vaccine IM 3 doses.</p> <p>Blood samples: Blood samples will be taken for research assays evaluating, humoral immunity, cell mediated immunity, and proteomics. See Blood Draw Table: Group 1 for timing of blood draws.</p> <p>Total blood volume for Group 1: approx 593 mL</p> </td></tr> <tr> <td data-bbox="373 1079 491 1396" style="text-align: center;">2</td><td data-bbox="491 1079 1519 1396"> <p>Vaccinations: Rabies vaccine ID 3 doses</p> <p>Blood samples: Blood samples will be taken for research assays evaluating humoral immunity, cell mediated immunity, and proteomics. See Blood Draw Table: Group 2 for timing of blood draws.</p> <p>Total blood volume for Group 2: approx 593 mL</p> </td></tr> <tr> <td data-bbox="373 1396 491 1717" style="text-align: center;">3</td><td data-bbox="491 1396 1519 1717"> <p>Vaccinations: Rabies vaccine IM 2 doses</p> <p>Blood samples: Blood samples will be taken for research assays evaluating humoral immunity, cell mediated immunity, and proteomics. See Blood Draw Table: Group 3 for timing of blood draws.</p> <p>Total blood volume for Group 3: approx 510 mL</p> </td></tr> </tbody> </table>	Group	Schedule of Vaccination and Blood Draw	1	<p>Vaccinations: Rabies vaccine IM 3 doses.</p> <p>Blood samples: Blood samples will be taken for research assays evaluating, humoral immunity, cell mediated immunity, and proteomics. See Blood Draw Table: Group 1 for timing of blood draws.</p> <p>Total blood volume for Group 1: approx 593 mL</p>	2	<p>Vaccinations: Rabies vaccine ID 3 doses</p> <p>Blood samples: Blood samples will be taken for research assays evaluating humoral immunity, cell mediated immunity, and proteomics. See Blood Draw Table: Group 2 for timing of blood draws.</p> <p>Total blood volume for Group 2: approx 593 mL</p>	3	<p>Vaccinations: Rabies vaccine IM 2 doses</p> <p>Blood samples: Blood samples will be taken for research assays evaluating humoral immunity, cell mediated immunity, and proteomics. See Blood Draw Table: Group 3 for timing of blood draws.</p> <p>Total blood volume for Group 3: approx 510 mL</p>
Group	Schedule of Vaccination and Blood Draw								
1	<p>Vaccinations: Rabies vaccine IM 3 doses.</p> <p>Blood samples: Blood samples will be taken for research assays evaluating, humoral immunity, cell mediated immunity, and proteomics. See Blood Draw Table: Group 1 for timing of blood draws.</p> <p>Total blood volume for Group 1: approx 593 mL</p>								
2	<p>Vaccinations: Rabies vaccine ID 3 doses</p> <p>Blood samples: Blood samples will be taken for research assays evaluating humoral immunity, cell mediated immunity, and proteomics. See Blood Draw Table: Group 2 for timing of blood draws.</p> <p>Total blood volume for Group 2: approx 593 mL</p>								
3	<p>Vaccinations: Rabies vaccine IM 2 doses</p> <p>Blood samples: Blood samples will be taken for research assays evaluating humoral immunity, cell mediated immunity, and proteomics. See Blood Draw Table: Group 3 for timing of blood draws.</p> <p>Total blood volume for Group 3: approx 510 mL</p>								

	4	<p><u>Vaccinations: Rabies vaccine ID 2 doses</u></p> <p><u>Blood samples:</u> Blood samples will be taken for research assays evaluating humoral immunity, cell mediated immunity, and proteomics. See Blood Draw Table: Group 4 for timing of blood draws.</p> <p>Total blood volume for Group 4: approx 510 mL</p>
	5	<p><u>Vaccination: Placebo IM 1 dose</u></p> <p><u>Blood Samples:</u> Blood samples will be taken for research assays evaluating proteomics. See Blood Draw Table: Group 5 for timing of blood draws.</p> <p>Total blood volume for Group 5: approx 9 mL</p>
	6	<p><u>Vaccination: Placebo IM 1 dose</u></p> <p><u>Blood Samples:</u> Blood samples will be taken for research assays evaluating proteomics. See Blood Draw Table: Group 6 for timing of blood draws.</p> <p>Total blood volume for Group 6: approx 9 mL</p>

Duration of the Trial:	The expected duration of a subject's participation in the trial will be 13 months for subjects in Groups 1, 2, 3, and 4. In groups 5 and 6 participation will end after Day 7.
Licensed Product:	RabAvert® Rabies Vaccine
Form:	RabAvert®: ≥2.5 units [contains albumin (human), amphotericin B (may have trace amounts), bovine gelatin, chicken egg protein, chlortetracycline (may have trace amounts), neomycin (may have trace amounts); PCEC; grown in chicken fibroblast culture; supplied with diluent].
Composition:	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 a sucrose density-gradient. The vaccine is lyophilized after addition of a stabilizer solution which 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. 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 μ g, 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.
Route:	After reconstitution the vaccine should be administered immediately. The potency of 1.0 ml of RabAvert Rabies vaccine is equal to or greater than 2.5 international units of rabies antigen. The Intramuscular (IM) dose is 1.0 ml and the intradermal (ID) dose is 0.1 ml.
Placebo:	Human Serum Albumin (HSA) Saline
Form:	Normal Saline with 0.03% human serum albumin added.
Composition:	Produced by Greer Labs, for use as a sterile diluent.
Route:	The Intramuscular (IM) dose is 1.0 ml and the Intradermal (ID) dose is 0.1 ml.
Inclusion Criteria:	Subjects must fulfill <i>all</i> of the following criteria in order to be eligible for trial enrollment: 1) Male and non-pregnant females aged \geq 18 to \leq 60 years on the day of inclusion 2) Able to comprehend and give informed consent 3) Able to attend all scheduled visits and to comply with all trial procedures 4) Subject in good health, based on medical history and physical examination

Exclusion Criteria	<p>A subject fulfilling <i>any</i> of the following criteria is to be excluded from trial enrollment:</p> <ol style="list-style-type: none"> 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 is well controlled is not an exclusion criteria. 6. Any history of cardiac arrhythmias, such as: Bradycardia, tachycardia, heart block, SVT, PAC, VF, VT, or any other conduction abnormalities. 7. Use of any immunosuppressive drug , including topical steroids of potency groups I, II or III within 30 days of the study period. 8. Any immunosuppressive disorder, such as HIV, common variable, active cancers or chemotherapy. 9. History of renal insufficiency or requiring dialysis. 10. 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. 11. Identified as an employee of the Investigator or study center, with direct involvement in the proposed study or other studies under the direction of that Investigator or study center, as well as family members (i.e., immediate, husband, wife and their children, adopted or natural) of the employee or the Investigator. <p>Temporary Exclusion Criteria: Moderate or severe acute illness/infection (according to investigator judgment) on the day of vaccination or febrile illness (temperature $\geq 38.0^{\circ}\text{C}$ [$\geq 100.4^{\circ}\text{F}$]). 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.</p>
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Statistical Methods:	<p>This will be a descriptive study to assess immunogenicity of a licensed rabies vaccine using test dosage schedules and routes of administration. No formal statistical hypothesis tests will be conducted. Descriptive analyses will be based on the per-protocol analysis sets.</p> <p>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 $\alpha=0.05$ and $\alpha=0.10$. When necessary, p-value corrections for multiple comparisons will be applied.</p> <p>Descriptive and any inferential analyses will be carried out using SAS Version 9.2 (or more recent versions), which is licensed and supplied by SAS Institute, Cary, NC, USA.</p> <p><i>Immunogenicity – Primary objectives:</i></p> <p>Immunogenicity against rabies virus will be assessed descriptively using the following parameters:</p> <p>Timepoints: Baseline, 28 days after initial dose of rabies vaccine and 7 days after a booster dose given at one year (372 days)</p> <ul style="list-style-type: none"> Number and percentage of subjects with a titer ≥ 0.5 IU/ml against rabies virus.
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Statistical Methods:	<p><i>Immunogenicity –Secondary objectives:</i></p> <p>Immunogenicity will be assessed descriptively using the following parameters:</p> <p>Time points: Baseline, 28 days and 365 days after initial vaccine dose</p> <p>Percentage of subjects in each group with titer of IgG antibodies above the protective limits (≥ 0.5 IU/ml) at 365 days, prior to boost.</p>
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Statistical Methods:	<p>Calculation of Sample Size:</p> <p>The planned sample size is 60 subjects, who will be randomly allocated (2:2:2:1:1) to four treatment groups and two control groups (i.e. 12 subjects in groups 1, 2, 3, and 4 and 6 subjects in groups 5 and 6). The sample size was chosen empirically, based on sample sizes of other exploratory studies of this general type. The study is not powered to test non-inferiority or equivalency in immunogenicity among groups according to vaccination dosage and route of administration.</p>
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Study Procedures: Group 1 (IM 3 doses)
 12 Subjects, 12 Visits, 13 Months Duration Per Subject

Study Day	-60 to 0	0	3	7	10	14	21	24	28	77	365	372
Visit Type	Screening	VAC BL	BL	VAC BL	BL	BL	VAC BL	BL	BL	BL	BOOST BL	BL
Time windows (days)	-60 to 0	0	+/-1	+/-1	+/-1	+/-1	+/-1	+/-1	+/-2	+/-7	+/-15	+/-15
Informed consent/IC review	X	X										
Inclusion/exclusion criteria	X	X		X			X				X	
Contraindications	X	X		X			X				X	
Significant medical history	X	X		X			X				X	
Physical exam	X											
Targeted PE		X	X	X	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X
Urine pregnancy test *Females	X*	X*		X*			X*				X*	
Concomitant therapy	X	X	X	X	X	X	X	X	X	X	X	X
Demography	X											
Randomization		X										
Blood Draw for Research Assays		X	X	X	X	X	X	X	X	X	X	X
Vaccination		X		X			X				X	
AE and SAE		X	X	X	X	X	X	X	X	X	X	X

Blood Draw Table: Group 1
Rabies vaccine Day 0, Day 7 and Day 21

Day	0	3	7	10	14	21	24	28	77	365	372
	VAC		VAC			VAC				Boost	
Antibody Assays/ Proteomics	4 x 8.5 ml	1 x 3 ml	2 x 8.5 ml	1 x 3 ml	2 x 8.5ml	2 x 8.5 ml	1 x 3 ml	2 x 8.5 ml	2 x 8.5 ml	4 x 8.5 ml	4 x 8.5 ml
CMI	8 x 8.0 ml	2 x 8.0 ml	6x 8.0 ml	2 x 8.0 ml	5 x 8.0 ml	5 x 8.0 ml	2 x 8.0 ml	5 x 8.0 ml	5 x 8.0 ml	8 x 8.0 ml	3 x 8.0 ml
Daily BV	98 ml	19 ml	65 ml	19 ml	57 ml	57 ml	19 ml	57 ml	57 ml	98 ml	58 ml
CBV (Total)	98 ml	117 ml	182 ml	201 ml	258 ml	315 ml	334 ml	391 ml	448 ml	546 ml	604 ml
Total (56 days)								391 ml	57 ml	98 ml	156 ml

Study Procedures: Group 2 (ID 3 doses)
 12 Subjects, 12 Visits, 13 Months Duration Per Subject

Study Day	-60 to 0	0	3	7	10	14	21	24	28	77	365	372
Visit Type	Screening	VAC BL	BL	VAC BL	BL	BL	VAC BL	BL	BL	BL	BOOST BL	BL
Time windows (days)	-60 to 0	0	+/-1	+/-1	+/-1	+/-1	+/-1	+/-1	+/-2	+/-7	+/-15	+/-15
Informed consent/IC review	X	X										
Inclusion/exclusion criteria	X	X		X			X				X	
Contraindications	X	X		X			X				X	
Significant medical history	X	X		X			X				X	
Physical Exam	X											
Targeted PE		X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X
Urine pregnancy test *Females	X*	X*		X*			X*				X*	
Concomitant therapy	X	X	X	X	X	X	X	X	X	X	X	X
Demography	X											
Randomization		X										
Blood Draw for Research Assays		X	X	X	X	X	X	X	X	X	X	X
Vaccination		X		X			X				X	
AE and SAE		X	X	X	X	X	X	X	X	X	X	X

Blood Draw Table: Group 2
Rabies vaccine Day 0, Day 7 and Day 21

Day	0	3	7	10	14	21	24	28	77	365	372
	VAC		VAC			VAC				Boost	
Antibody Assays/ Proteomics	4 x 8.5 ml	1 x 3 ml	2 x 8.5 ml	1 x 3 ml	2 x 8.5 ml	2 x 8.5 ml	1 x 3 ml	2 x 8.5 ml	2 x 8.5 ml	4 x 8.5 ml	4 x 8.5 ml
CMI	8 x 8.0 ml	2 x 8.0 ml	6 x 8.0 ml	2 x 8.0 ml	5 x 8.0 ml	5 x 8.0 ml	2 x 8.0 ml	5 x 8.0 ml	5 x 8.0 ml	8 x 8.0 ml	3 x 8.0 ml
Daily BV	98 ml	19 ml	65 ml	19 ml	57 ml	57 ml	19 ml	57 ml	57 ml	98 ml	58 ml
CBV (Total)	98 ml	117 ml	182 ml	201 ml	258 ml	315 ml	334 ml	391 ml	448 ml	546 ml	604 ml
Total (56 days)								391 ml	57 ml	98 ml	156 ml

Study Procedures: Group 3 (IM 2 doses)
 12 Subjects, 10 Visits, 13 Months Duration Per Subject

Study Day	-60 to 0	0	3	7	10	14	28	77	365	372
Visit Type	Screening	VAC BL	BL	VAC BL	BL	B L	BL	BL	BOOST BL	BL
Time windows (days)	-60 to 0	0	+/- 1	+/-1	+/-1	+/-1	+/-2	+/-7	+/-15	+/-15
Informed consent/IC review	X	X								
Inclusion/exclusion criteria	X	X		X					X	
Contraindications	X	X		X					X	
Significant medical history	X	X		X					X	
Physical Exam	X									
Targeted PE		X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X
Urine pregnancy test *Females	X*	X*		X*					X*	
Concomitant therapy	X	X	X	X	X	X	X	X	X	X
Demography	X									
Randomization		X								
Blood Draw for Research Assays		X	X	X	X	X	X	X	X	X
Vaccination		X		X					X	
AE and SAE		X	X	X	X	X	X	X	X	X

Blood Draw Table: Group 3
Rabies vaccine at Day 0 and Day 7

Day	0	3	7	10	14	28	77	365	372
	VAC		VAC					Boost	
Antibody Assays/ Proteomics	4 x 8.5 ml	1 x 3 ml	2 x 8.5 ml	1 x 3 ml	2 x 8.5 ml	2 x 8.5 ml	2 x 8.5 ml	4 x 8.5 ml	4 x 8.5 ml
CMI	8 x 8.0 ml	2 x 8.0 ml	5 x 8.0 ml	2 x 8.0 ml	5 x 8.0 ml	5 x 8.0 ml	5 x 8.0 ml	8 x 8.0 ml	3 x 8.0 ml
Daily BV	98 ml	19 ml	57 ml	19 ml	57 ml	57 ml	57 ml	98 ml	58 ml
CBV (Total)	98 ml	117 ml	174 ml	193 ml	250 ml	307 ml	364 ml	462 ml	520 ml
Total (56 days)						307 ml	57 ml	98 ml	156 ml

Study Procedures: Group 4 (ID 2 doses)
 12 Subjects, 10 Visits, 13 Months Duration Per Subject

Study Day	-60 to 0	0	3	7	10	14	28	77	365	372
Visit Type	Screening	VAC BL	BL	VAC BL	BL	B L	BL	BL	BOOST BL	BL
Time windows (days)	-60 to 0	0	+/- 1	+/- 1	+/- 1	+/- 1	+/- 2	+/- 7	+/- 15	+/- 15
Informed consent/IC review	X	X								
Inclusion/exclusion criteria	X	X		X					X	
Contraindications	X	X		X					X	
Significant medical history	X	X		X					X	
Physical Exam	X									
Targeted PE		X	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X	X	X
Urine pregnancy test *Females	X*	X*		X*					X*	
Concomitant therapy	X	X	X	X	X	X	X	X	X	X
Demography	X									
Randomization		X								
Blood Draw for Research Assays		X	X	X	X	X	X	X	X	X
Vaccination		X		X					X	
AE and SAE		X	X	X	X	X	X	X	X	X

Blood Draw Table: Group 4
Rabies vaccine at Day 0 and Day 7

Day	0	3	7	10	14	28	77	365	372
	VAC		VAC					Boost	
Antibody Assays/ Proteomics	4 x 8.5 ml	1 x 3 ml	2 x 8.5 ml	1 x 3 ml	2 x 8.5 ml	2 x 8.5 ml	2 x 8.5 ml	4 x 8.5 ml	4 x 8.5 ml
CMI	8 x 8.0 ml	2 x 8.0 ml	5 x 8.0 ml	2 x 8.0 ml	5 x 8.0 ml	5 x 8.0 ml	5 x 8.0 ml	8 x 8.0 ml	3 x 8.0 ml
Daily BV	98 ml	19 ml	57 ml	19 ml	57 ml	57 ml	57 ml	98 ml	58 ml
CBV (Total)	98 ml	117 ml	174 ml	193 ml	250 ml	307 ml	364 ml	462 ml	520 ml
Total (56 days)						307 ml	57 ml	98 ml	156 ml

Study Procedures: Group 5 (control)
6 Subjects, 4 Visits, 7 Day Duration Per Subject

Study Day	-60 to 0	0	3	7
Visit Type	Screening	VAC BL	BL	BL
Time windows (days)	-60 to 0	0	+/-1	+/-1
Informed consent/IC review	X	X		
Inclusion/exclusion criteria	X	X		
Contraindications	X	X		
Significant medical history	X			
Physical Exam	X			
Targeted PE		X	X	X
Vital signs	X	X	X	X
Urine pregnancy test *Females	X*	X*		
Concomitant therapy	X	X	X	X
Demography	X			
Randomization		X		
Blood Draw for Research Assays		X	X	X
Vaccination		X		
AE and SAE		X	X	X

Blood Draw Table: Group 5
Human Serum Albumin at Day 0

Day	0	3	7
	VAC		
Proteomics	1 x 3 ml	1 x 3 ml	1 x 3 ml
Daily BV	3 ml	3 ml	3 ml
CBV (Total)	3 ml	6 ml	9 ml

Study Procedures: Group 6 (control)
 6 Subjects, 4 Visits, 7 Day Duration Per Subject

Study Day	-60 to 0	0	3	7
Visit Type	Screening	VAC BL	BL	BL
Time windows (days)	-60 to 0	0	+/-1	+/-1
Informed consent/IC review	X	X		
Inclusion/exclusion criteria	X	X		
Contraindications	X	X		
Significant medical history	X	X		
Physical Exam	X			
Targeted PE		X	X	X
Vital signs	X	X	X	X
Urine pregnancy test *Females	X*	X*		
Concomitant therapy	X	X	X	X
Demography	X			
Randomization		X		
Blood Draw for Research Assays		X	X	X
Vaccination		X		
AE and SAE		X	X	X

Blood Draw Table: Group 6
Human Serum Albumin at Day 0

Day	0	3	7
	VAC		
Proteomics	1 x 3 ml	1 x 3 ml	1 x 3 ml
Daily BV	3 ml	3 ml	3 ml
CBV (Total)	3 ml	6ml	9 ml

List of Terms and Abbreviations

Ab	antibody
ADR	adverse drug reaction
AE	adverse event
AESI	adverse event of special interest
AR	adverse reaction
BL	blood sample visit
C	Celsius
CDM	Clinical Data Management
CI	confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CMI	Cell Mediated Immunity
CRA	Clinical Research Associate
CSTE	Council of State and Territorial Epidemiologists
D	day
eCRF	electronic case report form
EDC	electronic data capture
ELISA	enzyme linked immuno sorbent assay
F	Fahrenheit
FAS	full analysis set
GCI	Global Clinical Immunology
GCP	Good Clinical Practices
GM	geometric mean
GMT	geometric mean titer
GMTR	geometric mean titer ratio
HIV	human immunodeficiency virus

ICF	informed consent form
ID	intradermal
IM	intramuscular
IRB	Institutional Review Board
M	month
NR	not reportable
OD	optical density
PBMC	peripheral blood mononuclear cell
PDF	portable document format
PPAS	per-protocol analysis set
Product	Rabies vaccine
QA	quality assurance
R&D	Research and Development
RCDC	reverse cumulative distribution curve
RFFIT	rapid fluorescent focus inhibition test
SADR	Serious Adverse Drug Reaction
SAE	serious adverse event
SC	subcutaneous(ly)
SOP	standard operating procedure
UAR	unexpected adverse reaction
US	United States
V	visit
VAC	vaccination (with Rabies vaccine)
WRAIR	Walter Reed Army Institute of Research

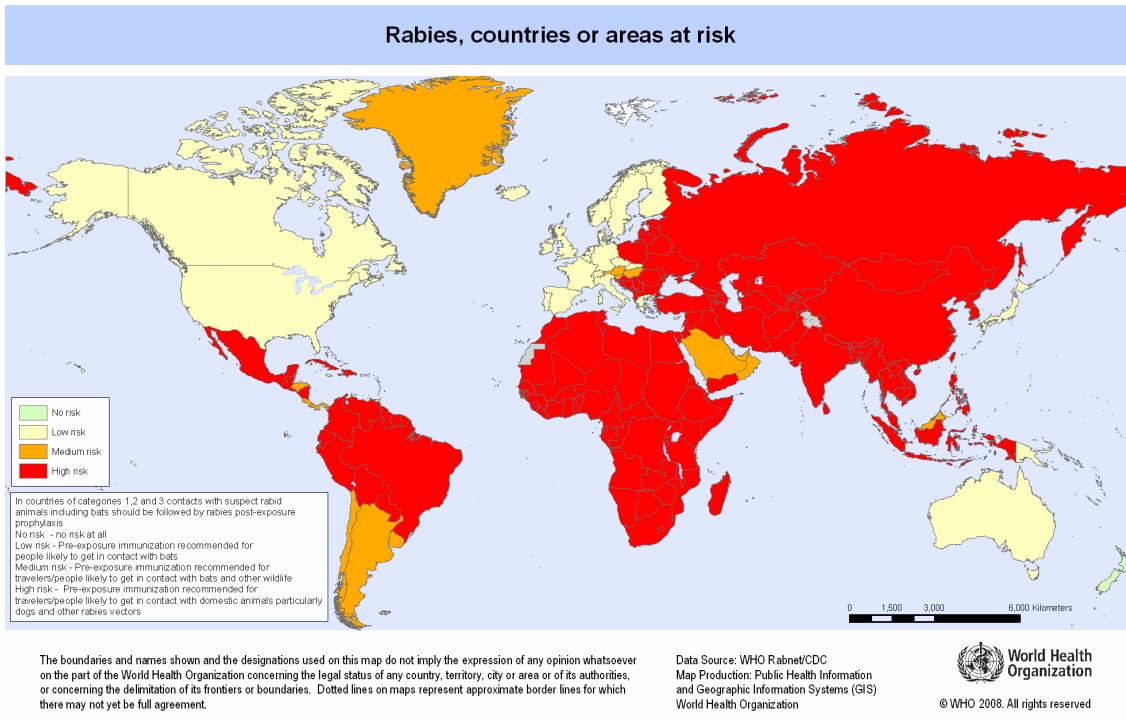
1 Introduction

This is an exploratory vaccine trial to evaluate immunogenicity of a non-licensed dosing schedule and route of administration for a currently FDA licensed rabies vaccine for pre-exposure prophylaxis against rabies infection. The goal of this study is to characterize the immune response and persistence of immunity to a shortened dose schedule and intradermal (ID) administration, relative to the current licensed dosing schedule of the rabies vaccine (3 dose (0, 7, 21 days) IM). Rabies virus is endemic throughout the world due to high rates of both wild and domestic animal rabies and the risk to deployed military in endemic areas is considerable. Currently the commonly supported pre-exposure prophylaxis regimen for rabies, in the United States is comprised of three, 1.0 ml intramuscular (IM) injections of the human diploid cell vaccine (HDCV) or purified chick embryo cell (PCEC) rabies vaccine on days 0, 7, and 21 or 28. Modified, two and three dose schedules of intradermal (ID) injections of 0.1 ml of HDCV and PCEC are the standard outside the US. These two and three dose intradermal schedules share a similar safety and immunogenicity profile to intramuscular vaccinations and are easily boosted at one year after vaccination. A death, this year, from rabies, of a US Soldier returned from Afghanistan underscores the importance of rabies pre-exposure prophylaxis for soldiers and the need to evaluate the safest, most effective means of vaccinating large deploying forces. While the current three dose, 1 ml IM rabies series is effective, a shortened, equally effective vaccination series with significantly smaller dose per injection would greatly improve the logistics and cost associated with universal or even targeted coverage of deploying soldiers. Evaluation of a shorter, smaller-dose, pre-exposure vaccination series for rabies is the goal of this study.

1.1 Background of Rabies

Rabies virus is a RNA virus, serotype 1 of 7 serotypes of the genus *Lyssavirus*, family *Rhabdovirus* (1). The lyssaviruses including rabies virus are found in a number of mammalian species and transmitted to humans as a zoonotic disease. The primary animal vector varies geographically. In North America: skunks, raccoons, foxes, bats are the primary animal vector; Western Europe: foxes and bats; Eastern Europe: foxes and dogs; Latin America: dogs and bats; Caribbean: mongooses; Africa: dogs, jackals, mongooses, and foxes; Asia: dogs, cats, monkeys, mongooses, and arctic foxes. The outer part of the virus protein contains the matrix protein and a glycoprotein on which are located the epitopes that induce neutralizing antibodies, components of the current vaccine, and responsible for protection from infection. Rabies is a global health problem with 55,000 deaths occurring every year (2). It is highly endemic in most areas of the world (figure 1). Considering that there is a highly effective pre and post-exposure prophylaxis regimen to rabies exposure, the global health problem is one of an access to care and effective rabies vaccines.

Figure 1. Global risk of rabies (World Health Organization, http://www.who.int/rabies/rabies_maps/en/index.html)



1.2 Background of the Licensed Product

A vaccine against rabies was the first vaccine to have been developed by Louis Pasteur; it was grown in and purified from rabbit spinal cords. The rabies vaccine that is currently available in the U.S. is derived from cell cultures, either a human diploid cell vaccine (HDCV) or as a purified chick embryo cell vaccine (PCECV) (1). Imovax Rabies (HDCV for pre or post-exposure) was developed by Sanofi Pasteur and licensed in 1980. RabAvert (PCECV for pre or post-exposure) was developed by Novartis and licensed in 1997. Table 1 demonstrates the current regimens for both pre and post-exposure prophylaxis with the rabies vaccine.

Table 1. Regimens for pre-exposure and post-exposure vaccination with rabies vaccines (1).

Vaccination, route	Days doses are given	Remark(s)
Preexposure		
im ^a	0, 7, and 21 or 28	Standard regimen
id ^b	0, 7, and 21 or 28	Economical, but not to be used in those people taking antimalarial medications
Postexposure^c		
im ^a	0, 3, 7, 14, and 28	US and WHO recommendations
im ^a	0 (2 doses), 7, and 21	Used in some countries when RIG is not indicated
id ^b	0, 3, 7 (2 doses each), 28, and 90	Used in Thailand with PVRV, PCECV, or PDEV
id ^b	0 (8 doses), 7 (4 doses), 28, and 90	Used in developing countries with cell culture vaccine
Booster dose (for reexposure)		
im ^a	0 and 3	Only after documented vaccination with cell culture vaccine ^d
id ^{b,d}	0 and 3	Only after documented vaccination with cell culture vaccine; ^d not recommended in the US

NOTE. Id, intradermally; PCECV, purified chick embryo cell culture vaccine; PDEV, purified duck embryo vaccine; PVRV, purified vero rabies vaccine; RIG, rabies immune globulin; WHO, World Health Organization.

^a Dose of 0.5 or 1 mL, depending on the vaccine given into the deltoid.

^b Dose of 0.1 or 0.2 mL, depending on the vaccine given over the deltoid.

^c Together with RIG.

^d Or demonstrated presence of virus-neutralizing antibodies after immunization with other vaccines.

The current rabies vaccines produce antibody response in 100% of individuals with durable immunity for years. In a prospective study over 10 years of follow-up, an antibody titer of 130 IU following vaccination indicated prolonged seropositivity with titers of 0.5 IU or greater considered protective (3). Pre-exposure vaccination is recommended for people who will be exposed to rabies virus either in the laboratory, environment or by occupation. It is recommended for travelers or global health workers who may have potential exposure (1). Reactions to the current rabies vaccines are mild with local pain, erythema, and swelling at the injection site. Systemic reactions are less frequent with headache and malaise (1). Guillain-Barré syndrome has been reported as a rare event following vaccination but its association with vaccination is uncertain (1). Allergic reactions were thought to result from the formation of antibodies to the human albumin stabilizer that is chemically altered by beta-propiolactone. Systemic reactions to newer vaccines with significantly lower concentrations of human albumin are rare (11). The current licensed delivery of the rabies vaccine is by the IM route. Due to the high cost of cell cultured vaccines strategies have been investigated to reduce the number of vaccine doses at an equivalent immune response by ID administration (4). Intradermal vaccination is thought to produce a better immune response as compared to IM delivery due to the antigen processing of Langerhans' cells of the epidermis. The ID dose is one-fifth of a dose of an IM delivery.

1.3 Potential Benefits to Subjects

Subjects Injected with Rabies Vaccine

Subjects will benefit from the rabies vaccine by developing protective antibody to the rabies virus.

1.4 Potential Risks to Subjects

Subjects Injected with Rabies Vaccine

Reactions to the current rabies vaccines as previously noted are mild with local pain, erythema, and swelling at the injection site. Systemic reactions are less frequent and include headache and malaise (1).

All subjects

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

1.5 Rationale

Antibody Assays: The rabies virus envelope glycoprotein G is the correlate for protective immunity and by World Health Organization (WHO) guidelines, an antibody titer for protection should be greater than or equal to 0.5 IU/ml. Several studies have demonstrated the high sensitivity and specificity of the Bio-Rad, enzyme-linked immunosorbent assay (ELISA), PLATELIA RABIES II® and correlation to the current reference method, rapid fluorescent focus inhibition test (RFFIT) (5, 6). The Bio-Rad Platelia Rabies II assay will be used to assess subjects' immune response to rabies vaccine.

Proteomics: Proteomics is the analysis of the expression, localizations, functions, and interactions of a set of proteins expressed by the genetic material of an organism under a given set of environmental conditions. This technique offers a novel method to evaluate clinical samples from experimental vaccination for identification of novel biomarkers and correlates of immunogenicity. For this protocol, the specific aim is to identify subtle changes in the immune response associated with rabies vaccination given at various dosing intervals and both ID and IM. It is likely this analysis can better characterize the immune response in individuals whose titer drops below 0.5 IU/mL and help determine if they are still protected as indicated by the rapid, robust return of high antibody titers with a single booster dose. The iTRAQ (isobaric technique for relative and absolute quantitation) proteomic method allows the quantitative profiling of proteins from complex mixtures and is referred to as multi-dimensional protein identification technology (MudPIT) (13-15) (7-9). Upstate Medical University Proteomics Core facility will be used to test samples and consists of an LTQ Orbitrap XL mass spectrometer (16-18). The core proteomics facility employs both iTRAQ labeling and 2D DIGE methods of sample preparation for protein isolation and identification. Ettan Spot Picker, Decyder 2D differential analysis software and Typhoon 9410 variable mode imager are available for the 2D DIGE samples. For this proposal, iTRAQ labeling will be conducted according to the manufacturer's protocol (Applied Biosystems). Initially, samples will undergo immunodepletion, using eluting columns to remove high abundance proteins that may overwhelm unique peptide signatures. Briefly, 100 µg of protein in 20 µl is denatured and reduced with denaturant (1µl) and reducing agent (2 µl) supplied with the kit. The samples are incubated for 1 hour at 60° C and cysteine blocking agent is added (1µl) followed by incubation at room temp for 10 min. The samples are digested with trypsin at 37° C overnight and then processed by adding iTRAQ reagents. The samples are mixed and incubated another hour at room temp. The separation and mass spectral analysis will be conducted as described (19). The labeled 4 samples are pooled together, diluted 1:10 with a cation exchange buffer A and are fractionated by SCX HPLC. The sample is loaded in buffer A and eluted with a gradient of 0-350 mM KCl over 40 min at 0.2 ml/min. Twenty fractions are collected and then desalting with a C18 zip tip prior to LC-MS/MS. Samples will be processed with a Orbitrap XL FT LC-MS-MS system. The fractions are

individually injected by autosampler onto a 5 μm C18 reverse phase column. The samples are eluted using a buffered gradient for 5 min. Nanospray data for MS and MS/MS are analyzed with ProQuant software as described (19) (10).

Cell Mediated Immunity (CMI)

We propose to monitor both the quality and quantity of immune activation immediately after vaccination as well the quality and quantity of the subsequently generated adaptive immunity in response to booster immunization with the RABVAX vaccine. Blood will be obtained for evaluation of cell-mediated immune responses to vaccination. At each time point blood will be collected in CPT tubes and PBMCs will be separated. The PBMCs will be stored frozen at -130C° or below until analyzed at SUNY Upstate Medical University or shipped to WRAIR for analysis. CMI assays will include ELISPOT assays and flow cytometry intracellular cytokine staining (ICS). Cryopreserved PBMC will be tested in batched format to determine the presence and frequency of vaccine responders and the magnitude of the immune response using IFN- γ spot-forming units as a read-out. Assays for T cell function will test at a minimum for IFN- γ production, however additional analysis for production of other cytokines such as TNF- α and IL-2, as well as the generation of short and long term effector and memory T cell populations can be performed in an identical manner using multiparameter flow cytometry. Flow cytometry will be used to comprehensively assess cellular activation and maturation in all lymphoid and myeloid cell lineages in PBMC, determine the functional profile of antigen-specific cells, and directly phenotype the antigen-specific cells.

2 Objectives

2.1 Primary Objectives

Immunogenicity

- 1) To describe percentage of subjects achieving a protective humoral immune response to each of the vaccine dosing schedules at day 28 and day 365 after first vaccine dose, and 7 days after a booster given at one year (372 days)

The endpoints for the primary objective(s) are presented in Section 16.1.

2.2 Secondary Objectives

Immunogenicity

- 1) To describe the persistence of the humoral immune response to each of the rabies vaccine routes 12 months post dose 1

2.3 Exploratory Objectives

Immunogenicity

- 1) Describe the cell-mediated immune response to rabies vaccination in groups 1-4.
- 2) Proteomic characterization of the host response before and after each vaccination to identify biomarkers and correlates of immunogenicity.

3 Investigators and Trial Organization

This trial will be conducted at Upstate Medical University (Upstate) in Syracuse, NY, US. The Principal Investigator and sub-investigators are from the Center for Global Health and Translational Science at Upstate Medical University. The trial team will be coordinated by Dr. Mark E. Polhemus.

Laboratory

Serology or antibody titer by RFFIT assessments will be performed by the Rabies Division, Kansas State University Laboratory. Exploratory assays will be performed at Division of Virology, Walter Reed Army Institute of Research, and the Department of Microbiology and Immunology, Upstate Medical University. Additional laboratories or contractors may be utilized in the development or conduct of some research assays.

4 Independent Ethics Committee / Institutional Review Board

Before the inclusion of the first subject, this protocol, the informed consent form(s) (ICF), subject recruitment procedures, and any other written information to be provided to subjects must be approved by Upstate's Institutional Review Board (IRB).

In accordance with Good Clinical Practice (GCP) and local regulations, each Investigator and / or the Sponsor are / is responsible for obtaining this approval before the start of the trial. If the protocol is subsequently amended, approval must be re-obtained for each amendment. 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 to the Sponsor.

The Investigator will submit written summaries of the status of the trial to the IRB annually, or more frequently if requested. All serious adverse events (SAEs) occurring during the trial that are related to vaccination will be reported by the Investigator to the IRB, according to IRB policy.

5 Trial Design

This will be a randomized, open-label, single center study conducted in 60 healthy adults aged ≥ 18 years to ≤ 60 years in the US.

Rabies vaccine will be administered to subjects in the four vaccination groups as follows:

Table 2: Schedules of vaccine administration and number of subjects by group

Vaccine Group	Day				Number per Group
	0	7	21	365	
1	IM	IM	IM	IM	12
2	ID	ID	ID	IM	12
3	IM	IM		IM	12
4	ID	ID		IM	12
5	IM control				6
5	ID control				6

Subjects will be randomly assigned to one of the six groups through a computer generated process. A standard permuted-block design will be used for randomization/allocation in order to avoid undesirable difference in the numbers of subjects assigned to each group.

Blood samples will be taken at each visit for research assays. Assays include evaluation of antibody assays, proteomics, evaluation and Cell Mediated Immunity. See section 5.3.

In all groups additional blood samples may be taken if required to assess AEs or abnormal laboratory findings. Additional biological samples (e.g., body fluid, tissue samples) may be collected in the event of an AE, or SAE. See section 14.1.2.

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.

5.1 Justification of the Trial Design

This exploratory study is designed to be a broad evaluation of the immune response, in healthy adults, to vaccination with rabies vaccine across a variety of vaccination scenarios. The goal is to characterize the host response in four vaccination schedules.

Evaluation will be focused on antibody response to trial regimens in relation to using the accepted rabies vaccination schedule. The goal is to evaluate host response to the delivery of a licensed vaccine using an ID delivery and a 2 dose schedule compared to the recommended vaccination schedule of 3 doses by IM delivery, using descriptive statistical methods and exploratory data analysis.

The placebo (human serum albumin) groups will have proteomic analyses performed. This will show any differences in the proteomic responses that are due to the mechanical differences in the different routes of administration.

5.2 Trial Plan

Eligible subjects will be identified and recruited. Each subject must sign and date the informed consent form before any procedure or treatment associated with the study is performed.

Depending on group assignment, subjects will receive vaccination in accordance with the schedule outlined in Table 2 above. There will be follow-up visits for review of adverse events and collection of blood samples.

Blood samples will be collected at Day 0 and at various time points throughout the study to test the immune response to rabies. Additional blood samples will be collected at different time points to evaluate the immune response to rabies vaccination. Additional biological samples (e.g. body fluids, tissue samples) may be collected in the event of an SAE.

5.3 Visit Procedures

Each of the six vaccination groups will each have a schedule of study procedures. These study procedures are described for each group separately below. Although the schedule of visits is different for each study group, the basic types of visit are the same and the same procedures will be followed.

VAC: vaccination visit at which the rabies vaccine is administered.

BL: blood sample visit at which a blood sample is obtained. These BL visits will occur at designated days or months after the previous administration of vaccine. If vaccination occurs on the same day, the blood sample will be obtained before vaccination.

All screening assessments must be completed within 60days prior to vaccination.

Screening numbers will be assigned when the subject signs the consent document starting with screening #100, 101, 102, etc. When the subject has completed the screening visit and is eligible and willing to continue participation, they will be randomized according to the randomization process outlined in section 11. Screen-fails will not be recorded in the data management system for the study. During the screening period, the following procedures and assessments will be carried out for each subject to determine their eligibility for participation in the study.

- Obtain informed consent
- Obtain medical history
- Record concomitant medications
- Record demographics
- Complete physical examination
- Review of inclusion and exclusion criteria
- Vital signs
- Urine pregnancy test (females of child bearing potential)

On Day 0, when the subject arrives for his/her first vaccination, the following events will occur before randomization:

- 1) Review informed consent
- 2) Review inclusion and exclusion criteria to confirm eligibility.
- 3) Review pertinent medical history Section 6.6 of the subject and any changes in medical history since screening.
- 4) Conduct a directed physical examination, as required Section 6.6, measure the subject's

temperature, and obtain vital signs.

- 5) For all women, obtain a urine sample and perform a urine pregnancy test prior to vaccination. Do not vaccinate if the test result is positive.
- 6) Review the subject's concomitant medications.
- 7) Record information in the source document. Concomitant medications and therapy will be recorded in the source document.

If the subject is eligible for vaccination they will be randomized to one of the six groups and then vaccination will begin on one of the following schedules, depending on randomization.

Group 1

Vacc Group	Day				No. per Group
	00	07	21	365	
1	IM	IM	IM	IM	12

Day 0 Rabies Vaccination #1

- 1) Confirm subject is randomized to Group 1.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females.
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for antibody assays, proteomics, CMI. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject rabies vaccine (intra-muscular injection in the deltoid).
- 9) Observe subject for 30 minutes, and record any adverse reaction(s) in the source document and the eCRF.

Day 3 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) Unsolicited AE/SAE assessment.

Day 7 Rabies Vaccination #2

- 1) Confirm subject is randomized to Group 1.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.

- 6) Obtain vital signs
- 7) Obtain blood samples for antibody assays, proteomics, CMI. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject rabies vaccine (intra-muscular injection in the deltoid).
- 9) Observe subject for 30 minutes, and record any adverse reaction(s) in the source document and the eCRF.
- 10) AE/SAE assessment.

Day 10 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 14 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 21 Rabies Vaccination #3

- 1) Confirm subject is randomized to Group 1.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for antibody assays, proteomics, CMI. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject rabies vaccine (intra-muscular injection in the deltoid).
- 9) Observe subject for 30 minutes, and record any adverse reaction(s) in the source document and the eCRF.
- 10) AE/SAE assessment.

Day 24 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 28 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) Unsolicited AE/SAE assessment.

Day 77 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 365 Rabies Boost

- 1) Confirm subject is randomized to Group 1.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females.
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for antibody assays, proteomics, CMI. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject rabies vaccine (intra-muscular injection in the deltoid).
- 9) Observe subject for 30 minutes, and record any adverse reaction(s) in the source document and the eCRF.
- 10) AE/SAE assessment.

Day 372 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Group 2

Vacc Group	Day				No. per Group
	00	07	21	365	
2	ID	ID	ID	IM	12

Day 0 Rabies Vaccination #1

- 1) Confirm subject is randomized to Group 2.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females.
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for antibody assays, proteomics, CMI. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject rabies vaccine (intradermal injection in the forearm).
- 9) Observe subject for 30 minutes, and record any adverse reaction(s) in the source document and the eCRF.

Day 3 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 7 Rabies Vaccination #2

- 1) Confirm subject is randomized to Group 2.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females.
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for antibody assays, proteomics, CMI. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject rabies vaccine (intradermal injection in the forearm). Observe subject for 30 minutes, and record any adverse reaction (s) in the source document and the eCRF.
- 9) AE/SAE assessment.

Day 10 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Directed history and physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 14 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 21 Rabies Vaccination #3

- 1) Confirm subject is randomized to Group 2.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females.
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for antibody assays, proteomics, CMI. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject rabies vaccine (intradermal injection in the forearm). Observe subject for 30 minutes, and record any adverse reaction (s) in the source document and the eCRF.
- 9) AE/SAE assessment.

Day 24 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 28 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 77 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 365 Rabies Boost

- 1) Confirm subject is randomized to Group 2.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females.
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for antibody assays, proteomics, CMI. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject rabies vaccine (intra-muscular injection in the deltoid). Observe subject for 30 minutes, and record any adverse reaction (s) in the source document and the eCRF.
- 9) AE/SAE assessment.

Day 372 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Vacc Group		Day		No. per Group
	00	07	365	
3	IM	IM	IM	12

Group 3**Day 0 Rabies Vaccination #1**

- 1) Confirm subject is randomized to Group 3.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females.
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for antibody assays, proteomics, CMI. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject rabies vaccine (intra-muscular injection in the deltoid).
- 9) Observe subject for 30 minutes, and record any adverse reaction(s) in the source document and the eCRF.

Day 3 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 7 Rabies Vaccination #2

- 1) Confirm subject is randomized to Group 2.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females.
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for antibody assays, proteomics, CMI. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject rabies vaccine (intra-muscular injection in the deltoid).
Observe subject for 30 minutes, and record any adverse reaction(s) in the source document and the eCRF.
- 9) AE/SAE assessment

Day 10 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 14 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 28 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 77 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 365 Rabies Boost

- 1) Confirm subject is randomized to Group 3.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females.
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for antibody assays, proteomics, CMI. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject rabies vaccine (intra-muscular injection in the deltoid). Observe subject for 30 minutes, and record any adverse reaction (s) in the source document and the eCRF.
- 9) AE/SAE assessment.

Day 372 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Group 4

Vacc Group	Day			No. per Group
	00	07	365	
4	ID	ID	IM	12

Day 0 Rabies Vaccination #1

- 1) Confirm subject is randomized to Group 4.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females.
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for antibody assays, proteomics, CMI. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject rabies vaccine (intradermal injection in the forearm).
- 9) Observe subject for 30 minutes, and record any adverse reaction(s) in the source document and the eCRF.

Day 3 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 7 Rabies Vaccination #2

- 1) Confirm subject is randomized to Group 4.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females.
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for antibody assays, proteomics, CMI. The blood samples must

be obtained before vaccination from the arm opposite to the one to be used for vaccination.

- 8) Inject rabies vaccine (intradermal injection in the forearm). Observe subject for 30 minutes, and record any adverse reaction (s) in the source document and the eCRF.
- 9) AE/SAE assessment.

Day 10 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 14 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 28 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 77 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 365 Rabies Boost

- 1) Confirm subject is randomized to Group 4.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females.
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for antibody assays, proteomics, CMI. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject rabies vaccine (intra-muscular injection in the deltoid). Observe subject for 30

Vacc Group		Day		No. per Group
	00	03	07	
5	IM			6

minutes, and record any adverse reaction (s) in the source document and the eCRF.

9) Observe subject for 30 minutes, and record any adverse reaction(s) in the source document and the eCRF.

10) AE/SAE assessment.

Day 372 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for antibody assays, proteomics and CMI.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Group 5

Day 0 Control Vaccination

- 1) Confirm subject is randomized to Group5.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females.
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for, proteomics. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject human serum albumin (intramuscular injection in the deltoid).
- 9) Observe subject for 30 minutes, and record any adverse reaction(s) in the source document and the eCRF.
- 10) AE/SAE assessment.

Day 3 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for proteomics.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 7 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for proteomics.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Group 6

Vacc Group		Day			No. per Group
		00	03	07	
6	ID				6

Day 0 Control Vaccination

- 1) Confirm subject is randomized to Group 6.
- 2) Review inclusion/exclusion criteria, including pregnancy test for females.
- 3) Targeted physical exam.
- 4) Concomitant medication review
- 5) Review medical history.
- 6) Obtain vital signs
- 7) Obtain blood samples for proteomics. The blood samples must be obtained before vaccination from the arm opposite to the one to be used for vaccination.
- 8) Inject human serum albumin (intradermal injection in the forearm).
- 9) Observe subject for 30 minutes, and record any adverse reaction(s) in the source document and the eCRF.

Day 3 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for proteomics.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

Day 7 post vaccination

- 1) Obtain vital signs.
- 2) Obtain blood samples for proteomics.
- 3) Targeted physical exam.
- 4) Concomitant medication review.
- 5) AE/SAE assessment.

5.4 Planned Trial Calendar

The following dates are approximate. The actual dates may differ as, for example, the trial will not start until all the appropriate regulatory and ethical approvals have been obtained.

February 2014: IRB Submission

Planned trial period - FVFS (first visit, first subject) to LCLS (last contact, last subject): October 2014, February 2016

October 2014: First subject, first vaccination

January 2015: Last subject, last vaccination

February 2016: Last contact, last subject/Final study visit

July 2016: Assays Complete

January 2017: Final clinical study report submission

6 Enrollment and Retention of Trial Population

6.1 Recruitment Procedures

Subjects will be recruited for participation in this clinical trial. The site will ensure that any advertisements used to recruit subjects (letters, pamphlets, posters, etc.) are submitted to the IRB for approval.

6.2 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 make 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 should be recorded in the source documents.

6.3 Screening Criteria

There are no screening criteria other than the inclusion and exclusion criteria.

6.4 Inclusion Criteria

Subjects must fulfill *all* of the following criteria in order to be eligible for trial enrollment:

- 1) Aged ≥ 18 to ≤ 60 years on the day of inclusion
- 2) Informed consent form has been signed and dated
- 3) Able to attend all scheduled visits and to comply with all trial procedures
- 4) Subject in good health, based on medical history and physical examination

^a “18 to 60 years” means from the day of the 18th birthday to the day before the 60th birthday

6.5 Exclusion Criteria

Subjects fulfilling *any* of the following criteria are to 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 is well controlled is not an exclusion criteria.
6. Any history of cardiac arrhythmias, such as: bradycardia, tachycardia, heart block, SVT, PAC, VT, VF, or any other conduction abnormalities.
7. Use of any immunosuppressive drug, including topical steroids of potency groups I, II or III within 30 days of the study period.
8. Any immunosuppressive disorder, such as HIV infection, common variable immunodeficiency, active cancers or chemotherapy.
9. History of renal insufficiency or requiring dialysis.
10. 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.

11. Identified as an employee of the Investigator or study center, with direct involvement in the proposed study or other studies under the direction of that Investigator or study center, as well as family members (i.e., immediate, husband, wife and their children, adopted or natural) of the employee or the Investigator.
12. Temporary Exclusion Criteria: Moderate or severe acute illness/infection (according to investigator judgment) on the day of vaccination or febrile illness (temperature $\geq 38.0^{\circ}\text{C}$ [$\geq 100.4^{\circ}\text{F}$]). 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.

6.6 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. Medical records will not be reviewed. 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

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

At subsequent visits, a history-driven physical examination will 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.

6.7 Contraindications for Subsequent Vaccinations

6.7.1 Temporary Contraindications

Should a subject experience one of the conditions listed below, the Investigator will postpone further vaccination until the condition is resolved.

- Ongoing clinical AE or biological abnormality related to the trial vaccination
- Febrile illness (temperature $\geq 38.0^{\circ}\text{C}$ [$\geq 100.4^{\circ}\text{F}$]) or moderate or severe acute illness/infection on the day of vaccination, according to Investigator judgment.

Postponement must still be within the timeframe for vaccination indicated in the Tables of Study Procedures. 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.

- Acute illness on day of vaccination that the investigator determines the subject to be ineligible for vaccine that day.

6.7.2 Definitive Contraindications

Should a subject experience one of the conditions listed below, the Investigator will discontinue vaccination:

- Pregnancy, as indicated by a positive urine test on vaccination days.
- An anaphylactic or other significant allergic reaction to the previous dose of vaccine
- Known or suspected congenital or acquired immunodeficiency; or receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 6 months; or long-term systemic corticosteroid therapy (prednisone or equivalent for more than 2 consecutive weeks within the past 3 months)
- SAE related to the study vaccine following the trial vaccination
- Participation in another clinical trial investigating a vaccine, drug, medical device, or medical procedure
- Deprived of freedom by an administrative or court order, or in an emergency setting, or hospitalized involuntarily. Subjects will not be withdrawn due to contraindication but will be followed up for safety and possibly immunogenicity assessment.

Note: If a subject who has already received at least 1 dose of the study vaccine, experiences a contraindication to further vaccination during the trial, they will continue to be followed for immunogenicity assessments and will not be discontinued from the trial; however, no additional vaccinations will be administered.

6.8 Other Conditions-for-Withdrawal beyond Definitive Contraindications

Subjects will be informed that they have the right to withdraw from the trial at any time.

A subject may be withdrawn from the study:

- At the discretion of the Investigator or Sponsor due to safety concerns without the subject's permission (withdrawal)
- At the request of the subject (dropout)

The following will result in automatic withdrawal or exclusion of a subject from the study:

- Significant noncompliance with the protocol, based on the Investigator's judgment

The reason for a withdrawal or dropout should be clearly documented in the source documents and on the eCRF.

Withdrawn subjects will not be replaced.

6.9 Lost to Follow-up Procedures

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.

6.10 Classification of Subjects Who Discontinue the Trial

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 18.
- **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 17.
- **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 6.9. 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.

6.10.1 Follow-up of Discontinuations

The site should complete all scheduled follow-ups with any subject who has prematurely terminated the trial because of an SAE, other type of AE, non-compliance with the protocol, or loss of eligibility, including definitive contraindications. Discontinued subjects will be followed up to 6 months after the last vaccination.

For subjects where the reason for early termination was lost to follow-up or if the subject withdrew informed consent and specified that they do not want to be contacted again and it is documented in the source document, the site will not attempt to obtain further information.

6.10.2 Follow-up and Reporting of Pregnancies

Pregnancy is an exclusion criterion for enrollment in this study, but a subject could potentially become pregnant during her participation. In case of pregnancy and if at least one dose of the study vaccines has been administered, the subject will not be discontinued from the trial and will

be followed for immunogenicity assessment. However, no additional vaccination will be administered.

All pregnancy cases should be reported if they occurred during the study. 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.

7 Safety Emergency Coverage

Subjects in the trial will be given contact numbers for the PI and study team members in the event of an emergency related to the trial. A study investigator will be available at all times for study related emergencies. If emergency health care is needed, subjects should seek care through the 911 system or local emergency services. Study team contact numbers should be used to notify the study team of an emergency after emergent health care issues have been handled.

8 Modification of the Trial and Protocol

Any amendments to this trial plan and protocol must be discussed with WRAIR. If agreement is reached concerning the need for an amendment, the PI will be responsible for completing the amendment and creating a new version of the protocol will replace the earlier version. All substantial amendments e.g. that affect the conduct of the trial or the safety of subjects, require IRB approval, and must also be forwarded to regulatory authorities.

An administrative amendment to a protocol is one that modifies some administrative or logistical aspect of the trial but does not affect its design or objectives or have an impact on the subjects' safety. Regulatory authorities need only be notified about administrative changes. Sites will be required to submit any administrative amendments to their IRB for notification and/or approval. The Investigator is responsible for ensuring that changes to an approved trial, during the period for which IRB approval has already been given, are not initiated without IRB review and approval, except to eliminate apparent immediate hazards to subjects.

9 Vaccines Administered

Subjects in Group 1 will receive 3 doses of Rabies vaccine administered IM at Days 0, 7,21 and 365.

Subjects in Group 2 will receive 3 doses of Rabies vaccine administered ID at Days 0, 7,21 and IM at Day 365.

Subjects in Group 3 will receive 2 doses of Rabies vaccine administered IM at Days 0,7 and 365.

Subjects in Group 4 will receive 2 doses of Rabies vaccine administered ID at Days 0,7, and IM at Day 365.

Subjects in Group 5 will receive 1 dose of Human Serum Albumin administered IM at Day 0.

Subjects in Group 6 will receive 1 dose of Human Serum Albumin administered ID at Day 0.

9.1 Identity of Trial Product: Rabies Vaccine

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 μ g, 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:	ID/IM
Batch number:	To be determined

9.1.1 Composition

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 a sucrose density-gradient. The vaccine is lyophilized after addition of a stabilizer solution which consists of buffered polygeline and potassium glutamate.

Placebo: Human Serum Albumin 0.03%. Produced by Greer Labs, for use as a sterile diluent.

Form:	Diluent
Dose:	1.0 ml of diluent
Route:	IM/ID
Batch/ Lot number:	To be determined

9.1.2 Preparation and Administration

The vaccine comes as powder and reconstituted for injection. Vaccine will be administered by ID in the forearm or IM injection in the deltoid.

9.1.3 Dose Selection and Timing

The dose for IM vaccinations will be 1 ml for each vaccination. The dose for ID vaccinations will be 0.1 ml for each vaccination. Two vaccination groups will receive vaccinations on Day 0, then Day 7, then Day 21. The other two vaccination groups will receive vaccinations on Day 0 and then Day 7.

9.1.4 Labeling and Packaging

Rabies Vaccine and Human Serum Albumin:

9.1.5 Product Shipment

Product will be purchased through the study team, who will be responsible for coordinating appropriate shipping and storage.

9.1.6 Product Storage

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

Vaccines will be kept in the Clinical Research Unit in a secure place with restricted access. Vaccines 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.

9.1.7 Product Accountability

Vaccine and human serum albumin accountability will be the responsibility of the study coordinator, who will maintain both accountability logs.

9.1.8 Replacement Doses

This is an open-label trial. In the case that a dose needs to be replaced, another vial should be removed from the site's supply.

9.1.9 Disposal of Unused Products

As the vaccine used in this study is an available licensed vaccine, any unused product may be used in an additional study conducted by the PI. Any unused human serum albumin can also be used in additional studies.

10 Blinding and Code-breaking Procedures

This is an open-label trial; no code breaking procedures need to be defined.

11 Randomization and Allocation Procedures

Each subject who meets the inclusion/exclusion criteria and signs an ICF will be assigned to one of the groups based on block randomization.

Table 2: Randomization

A statistician will prepare the randomization schedule to randomly assign subject numbers to group and treatment. An appropriate blocking scheme will be used to ensure that the balance between treatment assignments is maintained. The biostatistician from the Center for Research and Evaluation will provide master randomization list to programmer with database group and label preparers starting with subject ID numbers 0001 through 0060. No other study team members will have access to this list. The study team will not know the randomization sequence.

Once the screening process is complete and the subject is eligible to be enrolled in the trial, the subject ID number will be assigned by the clinical research coordinator or designee. Subject numbers will be assigned as a 4-digit subject number (0001, 0002, 0003, etc.). The first subject randomized will be 0001, etc.

Subjects will be enrolled in six groups. When a subject is enrolled into the study, they will be allocated to a particular group and complete all vaccinations and follow-ups according to the schedule for that group.

Subject identification numbers will not be reassigned for any reason.

12 Treatment Compliance

The following measures will ensure that the vaccine doses administered comply with those planned, and that any non-compliance is documented so that it can be accounted for in the data analyses:

- All vaccinations will be administered by qualified trial personnel.
- Study Coordinator will maintain accountability records of product delivery to the trial site, product inventory at the site, dose(s) given to each subject, and the disposal of unused or wasted doses.

13 Concomitant Medications or Other Therapies

At the time of enrollment, ongoing medications including other therapies should be recorded in the source document as well as new medications prescribed for new medical conditions/AEs during trial participation.

Documentation in the eCRF of concomitant medication will include all medications listed in the source documents.

Medications will be collected in the eCRF from the day of vaccination to the end of the follow-up period in general as they may impact the response to the vaccination and impact the

consistency of the information collected on concomitant medications at any vaccination.

Medications pertaining to the contraindications list will be reported throughout the course of the trial. The information reported in the eCRF for each reported medication will be limited to:

- Trade name
- Indication
- Start and stop dates

Dosage and administration route will not be recorded.

14 Management of Samples

See the Tables of Study Procedures and Section 5.2 for details of the sampling schedule.

14.1 Sample Collection

14.1.1 Blood Samples

See the Tables of Study Procedures and Section 5.2 for details of the sampling schedule.

Immediately prior to the blood draw, the staff member performing the procedure will verify the subject's identity; will obtain the pre-printed label and the sampling stage; and will attach the label to the tube. Blood is to be taken from the limb opposite to the one that will be used for injection of the Rabies vaccine.

14.1.2 Additional Biological Samples

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

14.2 Sample Preparation

An overview of the lab procedures is provided here.

Antibody Assays:

At each time point, blood samples will be obtained for measurement of neutralizing antibodies against rabies virus. The blood will be allowed to clot and the serum collected by centrifugation. The serum will be stored frozen at -70°C or below until shipment to WRAIR or partner laboratory for analysis.

CMI:

Blood will be obtained for evaluation of CMI responses to vaccination. At each time point blood will be collected and PBMCs will be separated. The PBMCs will be stored frozen at -70°C or below until analyzed at SUNY Upstate or shipment to WRAIR for analysis. CMI assays will include multiparameter flow cytometry, including intracellular cytokine staining, and possibly CFSE-based proliferation assays and epitope mapping depending on the results of initial testing.

Proteomics:

Blood samples will be obtained for analysis of the proteins expressed after vaccination with Rabies vaccine. Whole blood will be collected and processed in the proteomics core lab at Upstate Medical University.

14.3 Sample Storage and Shipment

During storage, serum tubes are to be kept in a freezer whose temperature is set and maintained at -20°C or below. The temperature will be monitored and documented on the appropriate form during the entire trial. If it rises above -10°C for any period of time, the Clinical Logistics Coordinator must be notified. See the Operating Guidelines for further details.

Shipments to the laboratories will be made only after appropriate monitoring, and following notification of the Clinical Logistics Coordinator. Sera will be shipped frozen, using dry ice to maintain them in a frozen state, in the packaging container provided by the carrier. Again, temperatures will be monitored. Shipments must be compliant with the International Air Transport Association (IATA) 602 regulations.

Samples will be shipped to the Walter Reed Army Institute of Research and Kansas State University.

14.4 Future Use of Stored Serum Samples for Research

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 in 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.

15 Clinical Supplies

The Investigator will supply all phlebotomy (with the exception of Vacutainers ®) and centrifugation equipment, including biohazard and/or safety supplies, blood collection tubes, cryotubes, cryotube storage boxes, cryotube labels, shipping containers. The biohazard and safety supplies include examination gloves, laboratory coats, and sharps disposal containers. The site will ensure that all biohazard wastes are disposed of in accordance with local practices. The Investigator will also supply appropriate space in a temperature-monitored refrigerator for the storage of the products and for the blood samples, and appropriate space in a temperature monitored freezer for serum and PBMC aliquots.

16 Endpoints and Assessment Methods

16.1 Primary Endpoints

16.1.1 Immunogenicity Endpoints

The primary endpoints for the evaluation of immunogenicity are:

- 1) Percentage of subjects in each group with titer of IgG antibodies above the protective limits (> or = 0.5 IU/ml) at 1 and 12 months after initial vaccine dose.

16.2 Secondary Endpoints

16.2.1 Immunogenicity Endpoints

- 1) Total IgG and neutralizing Ab levels, measured by ELISA and PRNT, against rabies virus at baseline and at 28 days 1 month and 365 days post-initial vaccine dose.

16.3 Assessment Methods

16.3.1 Immediate Post-vaccination Surveillance Period

Subjects will be kept under observation for 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.

17 Reporting AEs to IRBs

The Investigators will be responsible for informing the IRBs that reviewed the trial protocol of any reportable SAEs.

18 Reporting of Serious Adverse Events

In order to comply with current regulations on SAE reporting to health authorities, the Investigator must document all SAEs regardless of causal relationship, and notify the Sponsor within the notification timelines stated in the following sections. The Investigator will give access and provide the Sponsor and the local clinical research associate (CRA) with all necessary information to allow the Sponsor to conduct a detailed analysis of the safety of the investigational product. It is the responsibility of the Investigator to request all necessary documentation (e.g., medical records, discharge summary, autopsy) in order to provide comprehensive safety information. All relevant information must then be transcribed into the SAE form.

Timelines and process for SAE reporting

Initial reports of SAEs should be sent by the Upstate PI within 24 hours of the Investigator becoming aware of an SAE. In parallel, the Medical Monitor for the study should be notified. The Human Research Protection Office (HRPO) should also be notified via email: usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil Human Research Protection Office (HRPO), Office of Research Protections (ORP), U.S. Army Medical Research and Materiel Command, 810 Schreider Street Frederick, MD 21702-5012.

Process for SAE documentation

Following receipt of SAE reports, the Sponsor may raise additional questions to ensure complete documentation of the SAEs.

Timelines and process for pregnancy reporting

If the Investigator becomes aware on pregnancy of study participant, paper pregnancy reporting form should be completed. The Investigator will notify the medical monitor email: fazilit@upstate.edu and HRPO email: usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil Human Research Protection Office (HRPO), Office of Research Protections (ORP), U.S. Army Medical Research and Materiel Command, 810 Schreider Street Frederick, MD 21702-5012.

18.1 Follow-up Reporting by the Investigator

The SAE Form completed initially must be updated within 24 hours after the Investigator has become aware of any new relevant information concerning the SAE (e.g., outcome, precise description of medical history, results of the investigation). Copies of documents (e.g., medical records, discharge summary, autopsy) may be requested

The anonymity of the subject must always be respected when forwarding this information.

18.2 Reporting of SAEs Occurring After a Subject Has Completed the Study

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.

18.3 Assessment of Causality

The causal relationship between the SAE and the product will first be evaluated by the Investigator, using the following definitions:

0 - Not related: The AE is clearly / most probably caused by other etiologies such as an underlying condition, therapeutic intervention, or concomitant therapy; or the delay between vaccination and the onset of the SAE is incompatible with a causal relationship; or the SAE started before the first vaccination.

1 - Related: There is a “reasonable possibility” that the SAE was caused by the vaccination, meaning that there is evidence or arguments to suggest a causal relationship.

(ICH Guidelines, Clinical Safety Data Management E2A)

Following this, the Sponsor’s Product Safety Officer (PSO) will also assess the causal relationship to the product, based on the available information and current medical knowledge.

The decision to modify or discontinue the trial may be made after mutual agreement between the Sponsor and the Investigators.

18.4 Reporting SAEs to Health Authorities and IRBs

The Sponsor will inform the relevant health authorities of any reportable SAEs according to the local regulatory requirements. Reporting to the health authorities will be done according to the Sponsor’s SOPs.

The Sponsor’s Responsible Medical Officer will notify the Investigators in writing of the occurrence of any reportable SAEs. The Investigators will be responsible for informing the IRBs that reviewed the trial protocol of any reportable SAEs.

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.

19 Data

19.1 Data Collection and eCRF Completion

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. The eCRF has been designed specifically for this trial using a validated Electronic Records / Electronic Signature-compliant platform (21CFR Part 11).

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.

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.

19.2 Data Management

Management of Clinical Data

Data generated during the trial will be managed following two different processes:

- Clinical data, defined as all data reported in the eCRF, and laboratory data will be entered in the database held at Frontier Science in Amherst, NY.

During the trial, clinical data reported in the eCRFs will be integrated into the clinical

database under the responsibility of Upstate. 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 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 validation of the 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.

19.3 Data Review

A review of the data is anticipated through the data review process led by Data Management before database lock.

20 Statistical Methods

The analysis will be performed under the responsibility of Upstate Medical University in conjunction with the study partners.

This will be a descriptive study to assess immunogenicity of a licensed rabies vaccine using test dosage schedules and routes of administration. No formal statistical hypothesis tests will be conducted.. Descriptive analyses for the primary endpoints will be based on per-protocol analysis data sets using the data from blood samples taken at baseline (0 days), 28 days, and 365 days after the initial dose and at 7 days after a booster at one year (372 days).. Separate descriptive analyses will be conducted for each per-protocol data set and group.

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 $\alpha=0.05$ and $\alpha=0.10$. When necessary, p-value corrections for multiple comparisons will be applied.

A descriptive summary of adverse events that were reported throughout the trial will be produced. Descriptive and inferential analyses (if any) will be carried out using SAS Version 9.2 (or more recent versions), which is licensed and supplied by SAS Institute, Cary, NC, USA.

20.1 Statistical Methods for Primary Immunogenicity Objectives

Immunogenicity against rabies will be assessed descriptively using the following

parameters:

Timepoints: At baseline, 28 days and 372 days.

- Number and percentage of subjects with a titer ≥ 0.5 IU/ml against rabies virus by treatment group at days 28 and 372.

The descriptive statistics listed above will be calculated for each of the six groups for each blood draw. Descriptive results will be reported in tabular and graphic formats. A separate document, the statistical analysis plan (SAP) will be developed prior to data analysis. It will provide more detailed information about the specific descriptive statistics to be used and the format (tables, listings and figures) by which the statistics will be reported.

20.2 Statistical Methods for Secondary Immunogenicity Objectives

Immunogenicity will be assessed descriptively using the following parameters:

Timepoints: Day 28 and day 365.

The descriptive statistics listed above will be calculated for each of the six groups for the time points stated above. Specification of statistics and reporting formats will be provided in the SAP, as described above.

20.3 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. Three PP analysis sets will be produced for the primary objective that will contain separate data from blood drawn on day 0, day 28 and day 372, respectively. Descriptive analysis of the secondary endpoint will require a fourth per protocol analysis set for blood drawn on day 365. Subjects will be excluded from the PPAS for the following reasons:

- 1) Subject did not meet all protocol-specified inclusion/exclusion criteria or definitive contraindications (only for the second and third vaccination).
- 2) Subject did not receive the correct number of injections.
- 3) Administration of vaccine was not done as per protocol (site and route of administration).
- 4) Subject did not receive vaccine in the proper time window defined in the tables of the study procedures.
- 5) Subject did not provide a post-dose serology sample in the proper time window defined in the tables of the study procedures.

Subjects will remain in this population as long as they do not meet one of the above criteria, except for blood sampling timing and validity of the serology test result*. A PP set will be defined for each injection.

*Note: For example, a subject whose time period is outside the defined visit window only between the first injection and the blood sample taken after the first injection will be excluded from PP1 but may be kept in PP2 or PP3.

Subjects will be analyzed by the vaccine group to which they were randomized, subject to the exclusion criteria above

20.4 Full Analysis Set

The full analysis set (FAS) is defined as the subjects who received at least one injection of Rabies vaccine and had at least one blood sample drawn and valid post-injection serology result (i.e. a result different from “NR” or missing).

Subjects will be analyzed by the treatment group to which they were randomized.

20.5 Populations Used in Analyses

The immunogenicity analysis will be performed on the PP. The kinetic analysis will be performed on the FAS.

21 Handling of Missing Data and Outliers

21.1 Immunogenicity

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.

22 Determination of Sample Size and Power Calculation

The planned sample size is 60 subjects, who will be randomly allocated (2:2:2:2:1:1) to six treatment groups. 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 vaccination dosage and route of administration.

23 Ethical and Legal Issues and Investigator / Sponsor Responsibilities

23.1 Ethical Conduct of the Trial / Good Clinical Practice

This trial will be conducted in accordance with the Seoul revision of the Declaration of Helsinki as far as adopted by the concerned regulatory authorities, as well as in accordance with GCP as defined by International Conference on Harmonization (ICH), and 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.

23.2 Source Data and Source Documents

“Source data” are the data contained in source documents. Source documents are original documents or certified copies, and include, but are not limited to medical and hospital records, screening logs, informed consent / assent 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.

23.3 Confidentiality of Data and Access to Subject Records

Prior to initiation of the trial, the Investigator will sign a fully executed confidentiality agreement.

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

23.4 Monitoring, Auditing, and Archiving

23.4.1 Monitoring

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:

- The center has all the documents necessary for archiving.
- All samples have been shipped to the appropriate laboratories.
- All unused materials and products have been either destroyed or returned.

23.4.2 Audits and Inspections

A quality assurance (QA) audit may be performed at any time to verify that the trial has been conducted according to the protocol, GCP and ICH requirements, and other applicable regulations. An inspection may be conducted by regulatory authorities. The Investigator must allow direct access to trial documents during these inspections and audits.

23.4.3 Archiving

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

The Investigator will retain all documentation pertaining to the trial. 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. 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. All data and documents will be made available if requested by relevant authorities.

24 Stipends for Participation

Subjects will receive compensation for each study visit. Specifics of compensation will be included in the ICF and approved by the Upstate IRB.

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