

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

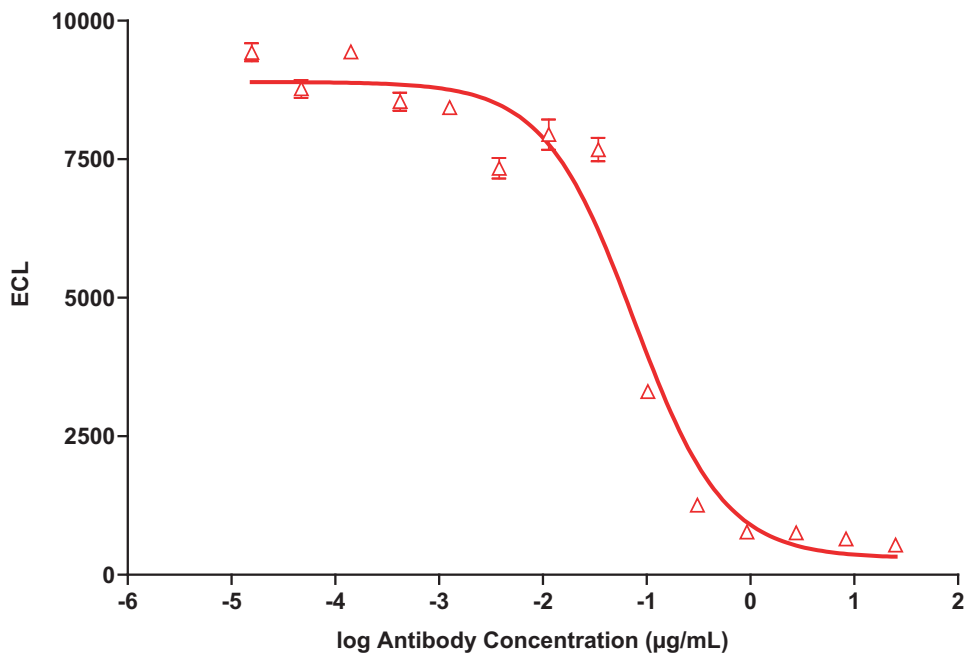
Supplement to: Migone T-S, Subramanian GM, Zhong J, et al. Raxibacumab for the treatment of inhalational anthrax. *N Engl J Med* 2009;361:135-44.

Supplemental information	Page
1. In vitro characterization	
1.1 Inhibition of PA-ATR binding	2
1.2 Kinetics of raxibacumab binding	3
1.3 Inhibition of cell killing	3
2. In vivo characterization	
2.1 Rat toxin model	5
3. Inhalational anthrax animal studies	
3.1 Aerosol challenge	7
3.2 Implantation of radiotelemetry	7
4. Assays	
4.1 Bacteremia (PCR)	8
4.2 Bacteremia (culture)	8
4.3 anti-PA	8
4.4 PA detection: screening and quantitative	9
4.5 Raxibacumab PK	9
4.6 Raxibacumab immunogenicity	9
4.7 TNA assay in animal serum	10
6. Anti-PA titer data	10
5. Inhalational challenge exposure data	11
6. Animal characterization (individual animal data)	11
7. Serum PA levels in rabbit and monkey studies	12
7. Histology (necropsy, histopathology, tissue bacteremia)	13
8. Summary of Human Studies.....	15
9. Tables for K-M plots to show subjects at risk over time	17
10. Statistical analysis	18

1.1 Inhibition of PA-ATR Binding

To measure raxibacumab inhibition of PA-ATR binding, biotinylated PA protein was preincubated with the antibody preparations. Purified flag-tagged ATR protein was then added and streptavidin-coated beads and anti-flag antibody labeled with ORI-TAG (IGEN International) were added. ECL was measured using an M8 ECL unit. The ECL data were analyzed using a 4-parameter logistic model. Different combinations of PA and ATR concentrations were tested to determine a PA-ATR combination that gave an optimal signal in the assay. A PA concentration of 300 ng/mL and an ATR concentration of 150 ng/mL were chosen to test the inhibitory activity of raxibacumab.

Raxibacumab was tested in the receptor-binding assay in the range of 0.0156 ng/mL to 25 μ g/mL. Raxibacumab inhibition of PA binding to ATR was potent and dose-dependent with an IC_{50} value of 503 pM.



Inhibition of PA binding to ATR with Raxibacumab: ECL was measured to determine the inhibition of PA binding to its recombinant soluble receptor, ATR protein. PA was pre-incubated with raxibacumab before adding to ATR. The assay was done in triplicate and data are presented as mean \pm SEM.

1.2 Kinetics of Raxibacumab Binding Analyzed by BIAcore

For the BIAcore studies, PA was immobilized on individual flow cells of a BIAcore CM5 sensor chip. The raxibacumab was serially diluted from 50 $\mu\text{g/mL}$ (333 nM) to 0.625 $\mu\text{g/mL}$ (4.1 nM) and placed in contact with the proteins during a 4-minute association phase. The off rate of raxibacumab was determined by washing the complex in the presence of buffer for 5 minutes. The binding data were analyzed using the BIA evaluation software, Version 3.1, with the results summarized. Raxibacumab showed high affinity binding to PA with an equilibrium binding constant (K_d) of 2.78 nM.

Binding kinetics of raxibacumab to PA

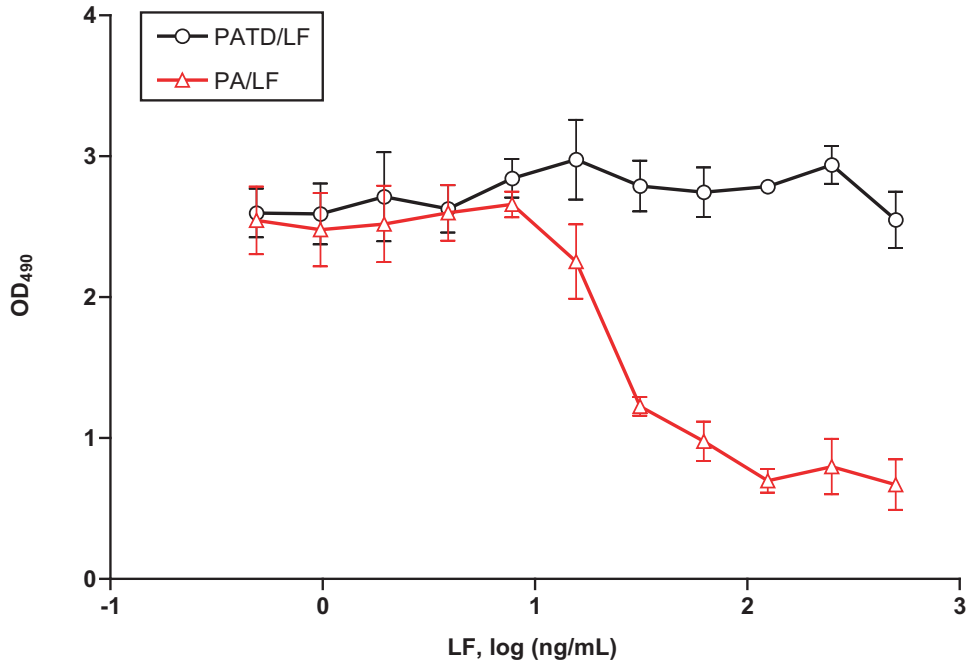
<u>Raxibacumab</u>	<u>k_a (1/Ms)</u>	<u>k_d (1/s)</u>	<u>K_d (M)</u>
Run 1	9.10×10^4	3.16×10^{-4}	3.47×10^{-9}
Run 2	2.47×10^5	8.64×10^{-4}	3.50×10^{-9}
Run 3	2.60×10^5	4.83×10^{-4}	1.86×10^{-9}
Mean	1.99×10^5	5.54×10^{-4}	2.78×10^{-9}

k_a (1/Ms), association rate constant; k_d (1/s), dissociation rate constant;
 K_d (M) = k_a/k_d , equilibrium binding constant.

1.3 Inhibition of Cell Killing

The ability of raxibacumab to inhibit cell killing caused by lethal toxin (PA/LF) was evaluated using the J774A.1 murine macrophage cell line. The cells were seeded in a 96-well micro titer plate and incubated overnight. The next day, fresh medium containing LF was added. To detect viable cells after lethal toxin treatment, CellTiter 96 AQueous One Solution Reagent (Promega) was incubated with the cells. Plates were then read at 490 nm using SpectraMax250. CellTiter 96 AQueous One Solution Reagent contains a tetrazolium compound that is reduced by metabolically active cells into a colored formazan product that can be measured by absorbance at 490 nm.

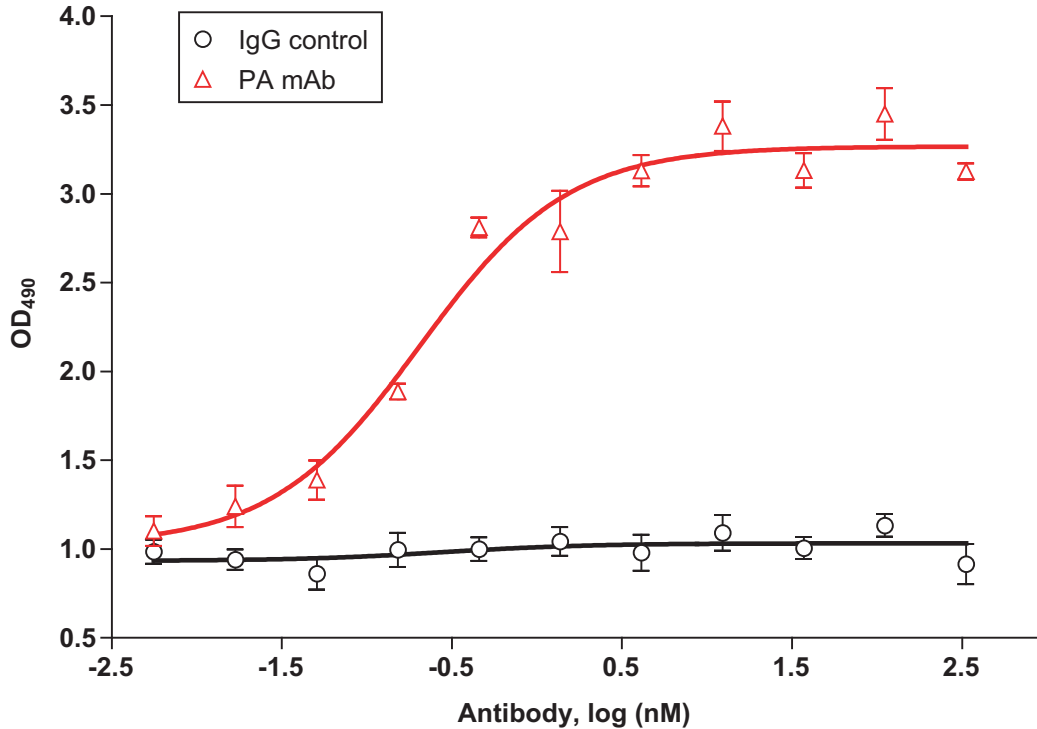
Different concentrations of LF were tested to determine the optimal amount of LF in the lethal toxin to use in the J774A.1 cell killing assay. Increasing concentrations of LF mixed with PA resulted in J774A.1 cell killing, while LF in the presence of translocation defective PA/LF did not. Based on these results, 50 ng/mL LF was chosen for subsequent assays with PA and raxibacumab.



Titration of lethal factor in J774A.1 cell killing assay

Different concentrations of LF were tested in the presence of 100 ng/mL PA. The starting concentration of LF was 500 ng/mL and 2x serial dilutions were made for the assay. PATD protein was used as a negative control. The assay was done in triplicate and data are presented as mean ± SEM.

The ability of raxibacumab to inhibit killing then was compared with a negative control IgG1 antibody in the cell killing assay. Raxibacumab inhibited lethal toxin-mediated cell death with complete protection at higher antibody concentrations, while the negative control antibody did not. The IC₅₀ for raxibacumab was 0.21 nM in this assay.



Inhibition of lethal toxin-induced cell killing by raxibacumab

Serial dilutions of raxibacumab (PA mAb) or negative control IgG antibody were pre-incubated with PA, and the mixture was added with LF to J774A.1 cells to induce killing. The assay was performed in triplicate and data are presented as mean ± SEM

2. Rat toxin model: SC, IM and IV Administration of Raxibacumab 24 Hours Prior to Injection of Lethal Toxin

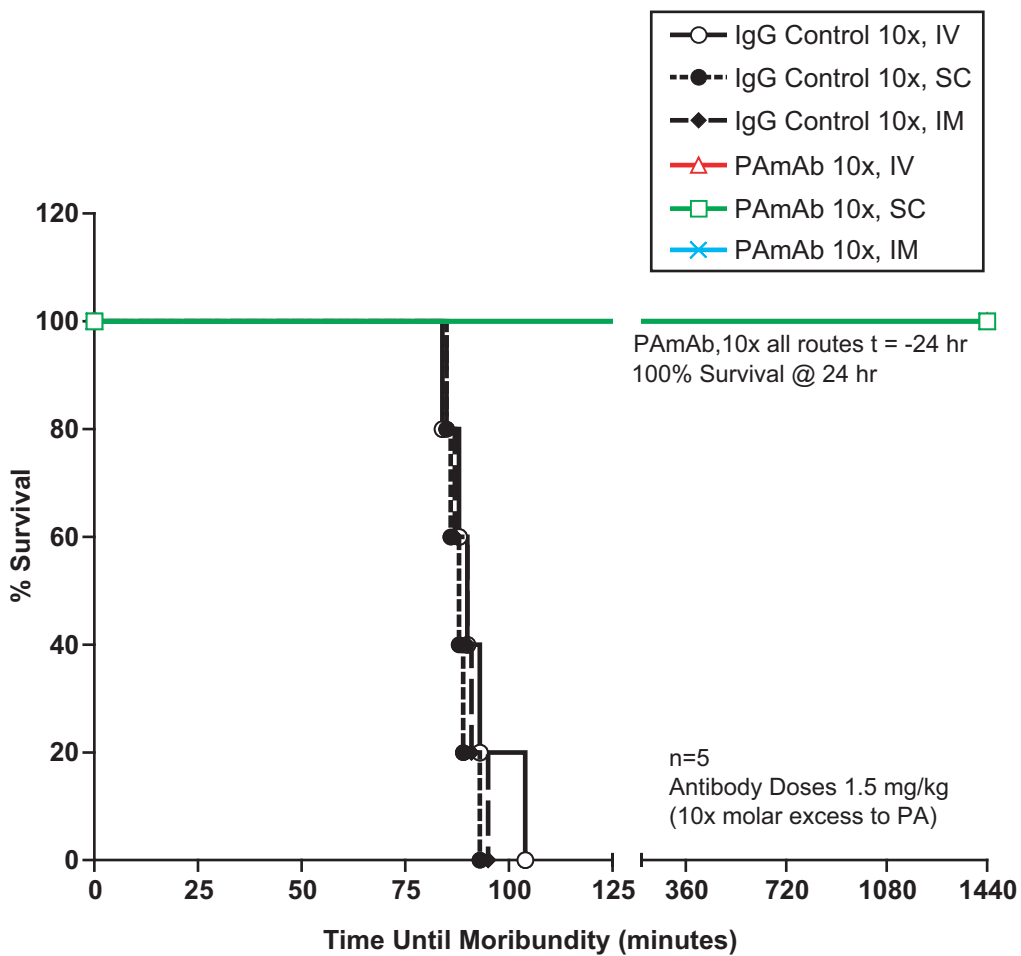
Male Fisher 344 rats were divided into 6 groups of 5 animals. All animals were injected under isoflurane anesthesia. The rats received a penile vein injection of lethal toxin (t = 0 minutes) and a SC, IM, or IV injection of 1.5 mg/kg raxibacumab or control IgG 24 hours before the lethal toxin injection (t = -24 hours).

Rat toxin challenge model; SC, IM or IV administration of antibody at t = -24 hours

<u>Group</u>	<u>n</u>	<u>IgG Control (mg/kg)</u> <u>(Fold Excess to PA)</u>	<u>Raxibacumab (mg/kg)</u> <u>(Fold Excess to PA)</u>	<u>Route of mAb</u> <u>Injection</u>	<u>PA</u> <u>(µg/rat)</u>	<u>LF</u> <u>(µg/rat)</u>
A	5	-	1.5 (10x)	SC	22.5	8
B	5	-	1.5 (10x)	IM	22.5	8
C	5	-	1.5 (10x)	IV	22.5	8
D	5	1.5 (10x)	-	SC	22.5	8
E	5	1.5 (10x)	-	IM	22.5	8
F	5	1.5 (10x)	-	IV	22.5	8

A single IV, SC, or IM injection of raxibacumab 24 hours prior to lethal toxin injection provided 100% survival at 24 hours. In contrast, a single injection of the negative control mAb, regardless of route of administration, provided no protection with 0% survival.

There is also a significant difference in the TTM among the rats in these treatment groups (p -value < 0.0001). Compared with the rats in the IgG control groups, all rats in the raxibacumab treated groups had a significantly longer TTM than those in the IgG control treated groups in the same injection route (all p -values = 0.0018). All rats in the raxibacumab treated groups survived the study while rats in the IgG control groups died with a median time of 90 minutes.



Effect of raxibacumab on survival of Fisher 344 rats challenged with lethal toxin; IV, SC or IM administration of antibody at $t = -24$ hours.

Antibodies were given IV, SC, or IM 24 hours prior to the lethal toxin challenge. Antibody doses were 1.5 mg/kg (10x molar excess to PA). There is a significant difference in the survival time among the rats in these treatment groups (p -value < 0.0001). Compared with the rats in the IgG control groups, all rats in the raxibacumab (PA mAb) treated groups had a significantly

longer TTM than those in the IgG control treated groups in the same injection route (all p-values = 0.0018).

Effect of raxibacumab on TTM of Fisher 344 rats challenged with lethal toxin, SC, IM or IV administration of antibody at t = -24 hours

<u>Treatment/Route</u>	<u>n</u>	<u>Deaths</u>	<u>TTM (minutes)</u>	<u>P Value^a</u>
IgG control, 1.5 mg/kg SC	5	5	88	-
IgG control, 1.5 mg/kg IM	5	5	90	-
IgG control, 1.5 mg/kg IV	5	5	90	-
Raxibacumab, 1.5 mg/kg SC	5	0	> 1440	0.0018
Raxibacumab, 1.5 mg/kg IM	5	0	> 1440	0.0018
Raxibacumab, 1.5 mg/kg IV	5	0	> 1440	0.0018

^aFrom a log-rank test for the comparison vs IgG control 1.5 mg/kg group at the same injection route.

The data from this study demonstrate that raxibacumab, regardless of route of administration, provides complete protection against mortality when given 24 hours before anthrax lethal toxin challenge.

3. Spore challenge experiments

3.1 Aerosol Challenge

Monkeys were transported into the BL-3 ten to fourteen days prior to challenge to allow time for acclimation. Monkeys were anesthetized with Telazol (1-6 mg/kg, IM) and placed into a plethysmography chamber and a Class III cabinet system. Monkeys were aerosol-challenged with a targeted 200 LD₅₀ [1.24×10^7 spores as 1 LD₅₀ is defined as 61800 spores] dose (Vasconcelos, et. al., 2003) of *B. anthracis* spores (Ames strain) aerosolized by a Collison nebulizer and delivered via a head-only inhalation exposure chamber.

Rabbits were transported into the BL-3 seven days prior to challenge to allow time for acclimation. Rabbits were placed individually into a plethysmography chamber and a Class III cabinet system, and aerosol challenged with a targeted 200 LD₅₀ [2.1×10^7 spores] inhaled dose of *B. anthracis* (Ames strain) spores. The Ames LD₅₀ value (105,000 colony forming units [cfu]) published from USAMRIID (Zauch, 1998) was used. The aerosol challenge duration will be based upon an estimated aerosol challenge concentration and a cumulative minute volume gathered "real" time throughout the exposure. Aerosol concentrations of *B. anthracis* will be quantified by determination of cfu. Effluent streams will be collected directly from an animal exposure port by an in-line impinger (Model 7541, Ace Glass Incorporated). Serial dilutions of impinger samples will be plated and enumerated.

3.2 Surgical Procedure for the Implantation of a Radiotelemetry Transmitter in the Monkey.

Once anesthetized, the monkeys were prepared for aseptic surgery: a midline incision was made in the skin overlying the abdominal cavity and the cavity was entered via an incision along the linea alba. The transmitter body was inserted into the abdomen and attached to the left lateral wall of the abdominal cavity with nonabsorbable suture. All abdominal muscular incisions were closed with absorbable suture. All skin incisions were closed with absorbable sutures and Vetbond (tissue adhesive) applied. Appropriate doses of antibiotics, analgesics and anti-inflammatory drugs were administered during the course of post-operative care.

4. Assays

4.1 Bacteremia (PCR)

Genomic DNA was isolated from 100 μ l of blood using a modified version of the QIAGEN DNeasy Kit. The Taqman analysis was performed using a ABI Prism 7700 Sequence Detector. Specific sets of primers and probes for PA (pagA) and for rabbit or monkey 18S were used for each sample. Taqman is quantitative PCR that uses a forward primer, a reverse primer and a probe. The probe is a primer that has a fluorescent dye at the 5 prime end and a quencher dye at the 3 prime end. The instrument uses laser light directed into each reaction well and measures the fluorescence emitted from the fluorescent dye. When the probe is intact the energy from the fluorescent dye is absorbed by the quencher dye thus no light is emitted from the well. As the PCR amplification progresses the exonuclease activity of the Taq enzyme digests the probe allowing the fluorescent and quencher dyes to drift apart permitting the fluorescence to escape the well. The resulting data is shown as a graph of fluorescence vs cycle number with a Ct (Cycle threshold) value given for each sample. The Ct value is the cycle number at which the sample's fluorescence crosses a pre-selected fluorescence threshold. The fluorescence threshold used is the same for all samples in the plate. The greater the number of starting templates in the Taqman reaction the sooner it will cross the fluorescence threshold and the lower the Ct value will be. A Ct value of 40 means the fluorescence never reached the fluorescence threshold. For rabbits, an animal was considered positive if it had a Ct value of less than 38; for monkeys, an animal was considered positive if it had a Ct value of less than 36.

4.2 Bacteremia (Culture)

To determine bacteremia status of aerosol challenged animals, whole blood is streaked for isolation on blood agar plates (or other appropriate solid media) and incubated at $\sim 37^{\circ}\text{C}$ for a minimum of 48 hours. Following incubation macroscopic observations are performed to assess the presence or absence of bacterial colonies. Colonies are assessed for hemolysis type, size, color, and morphology. If γ -hemolytic, white colonies, 4-10mm in diameter with a rough appearance and irregular edges are present the sample is documented as positive for Bacillus bacteremia. A sterile solid media plate is incubated at $\sim 37^{\circ}\text{C}$ and used as a negative control plate.

4.3 anti PA assay

ELISA plates are coated with Wild Type Protective Antigen (PAWT). Each plate contains a standard curve made up of 9-3 fold serial dilutions starting at 300ng/ml,

negative and positive controls, and up to 7 test serum samples. The controls and samples are analyzed in triplicate with the samples diluted to 1:100, 1:500, and 1:5000. The controls and test samples are incubated in the respective wells of coated plates to enable the capture of PA-specific antibodies from serum on the plate. The capture antibodies are then detected by addition of HRP-conjugated goat anti-human IgG (H+L). The bound HRP activity is quantitated by the color conversion of the TMB substrate in the presence of hydrogen peroxide. The absorbance is measured at 450 nm after stopping the reaction with dilute acid. The anti-PA antibody concentrations in the samples are then back calculated from the standard curve. LOQ is 0.6ug/mL in monkey serum and 1.25ng/mL in rabbit plasma

4.4 PA assay- screening and quantitative

The screening assay is an electrochemiluminescence-based assay. Undiluted serum samples in triplicate wells are incubated in the presence of rabbit anti-PA-biotin (capture) and rabbit anti-PA-sulfo-tag (detector) for 2 hours at 37°C in a streptavidin-coated assay plate on a plate shaker. Following this incubation, plates are washed, and read for electrochemiluminescence counts. LOQ is 1ng/mL in monkey serum and 0.4ng/mL in rabbit serum

The quantitative assay is an electrochemiluminescence-based assay. Undiluted serum samples in duplicate wells are incubated with rabbit anti-PA-biotin (capture) and rabbit anti-PA- sulfo-tag (detector) and allowed to equilibrate for 4 hours at 37°C in a streptavidin-coated assay plate. Following equilibration, plates are washed, and read for electrochemiluminescence counts. The counts of the samples are interpolated off of a PA standard curve prepared in neat pooled rabbit serum. These values are then multiplied by the dilution factor for a final concentration. LOQ is 0.65ng/mL in monkey serum and 0.34ng/mL in rabbit serum

4.5 Raxibacumab PK assay

A capture protein, biotinylated-PA, is bound to a MSD streptavidin-coated multi-array 96-well assay plate. The Raxibacumab in serum samples binds to the biotinylated-PA and is detected by the addition of MSD SULFO-TAG™ goat anti-human antibody, an electrochemiluminescent label. MSD Read Buffer is added to the plate and the plate is inserted into a MSD plate reader where voltage applied to the plate electrodes causes MSD SULFO-TAG™ goat anti-human antibody bound to Raxibacumab to emit light. Electrochemiluminescence is a measurement of Raxibacumab present in the serum. The concentration of Raxibacumab in serum samples is interpolated from a reference Raxibacumab standard curve. LOQ is 0.5ug/mL in human serum, 0.94ug/mL in monkey serum and 0.75ug/mL in rabbit serum

4.6 Raxibacumab Immunogenicity assay

Serum samples are assessed for anti-raxibacumab activity in Assay A, the screening assay. An electrochemiluminescence signal threshold (cut point) was established in the qualification experiments, above which samples are considered potential positive. If a potential positive sample is identified in Assay A, it is further tested in Assay B, the inhibition of binding assay, to confirm the specificity of binding. If the specificity is confirmed, the sample is considered anti-raxibacumab positive.

The screening assay is an electrochemiluminescence-based bridging assay. Undiluted serum samples in duplicate wells are acidified with hydrochloric acid for 1 hour to dissociate raxibacumab from anti raxibacumab antibodies. Acidified serum samples are then neutralized in the presence of raxibacumab-biotin (capture) and raxibacumab-sulfo-tag (detector) and allowed to re-equilibrate for 4 hours at room temperature in a streptavidin-coated assay plate. Following re-equilibration, plates are washed once, and read for electrochemiluminescence counts. LOD is 0.1ug/mL in human serum

4.7 TNA assay in animal serum

This assay to measure the host's toxin neutralizing antibodies was based on a published procedure (Hering 2004). This assay is based on the principle that neutralizing antibodies to protective antigen (PA) bind to, and prevent, PA from shuttling another anthrax toxin component, lethal factor (LF, a bacterial metalloprotease), into target J744A.1 macrophages. In the absence of neutralizing antibodies, LeTx complexes enter the cells causing cell death. Inhibition of PA binding is correlated with increased cell viability and is used to determine the relative titer of serum samples compared to a known positive control. LOD is a titer=52.

5. Anti PA titers in surviving animals:

Of the 30 raxibacumab-treated monkeys in the prophylaxis study, 22 animals survived the spore challenge and had serum samples re-drawn 6 months later to analyze the immune response generated. All the animals had elevated titers compared to the pre-challenge draw. The animals were then re-challenged (11 months after the first challenge) and anti-PA titers again tested 28 days post-second challenge. The magnitude of titers measured at this timepoint is also due to the presence of high raxibacumab concentrations in the samples.

Sample ID	raxibacumab treatment		<u>Anti-PA ELISA</u>		
	Group	Gender	Anti-PA titer (Pre-Challenge)	Anti-PA Titer 6 months after initial challenge	Anti-PA titer 28 days after second challenge
16881	10 mg/kg	F	217	40283	380092
16940	10 mg/kg	F	140	49597	445782
16675	10 mg/kg	F	2267	17074	187801
16642	10 mg/kg	F	480	48297	211124
17263	10 mg/kg	M	893	6468	N/A ¹
16726	10 mg/kg	M	386	6064	119697
17259	20 mg/kg	M	542	34506	244443
16904	20 mg/kg	F	399	13670	196570
17270	20 mg/kg	M	373	30894	316710
17261	20 mg/kg	M	558	9116	348454
17284	20 mg/kg	M	801	12612	551115
17242	20 mg/kg	M	128	3524	96388

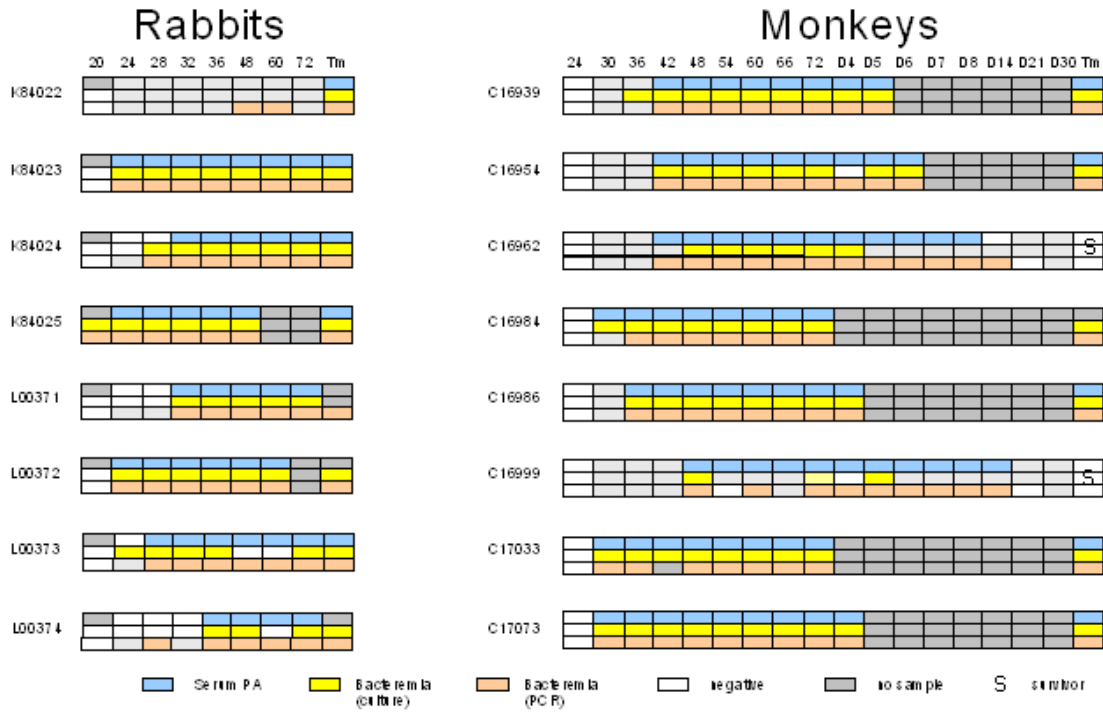
16678	20 mg/kg	F	192	4622	443091
16620	40 mg/kg	F	567	27970	789865
17245	40 mg/kg	M	886	27448	570964
16796	40 mg/kg	F	182	9484	251179
16871	40 mg/kg	F	169	10969	122282
16864	40 mg/kg	F	1976	22997	357606
17279	40 mg/kg	M	207	3890	148999
16676	40 mg/kg	F	2902	13493	403768
16698	40 mg/kg	M	195	9361	487368
16716	40 mg/kg	M	270	10536	123234

1 Animal died prior to second challenge due to an illness deemed not related to the study

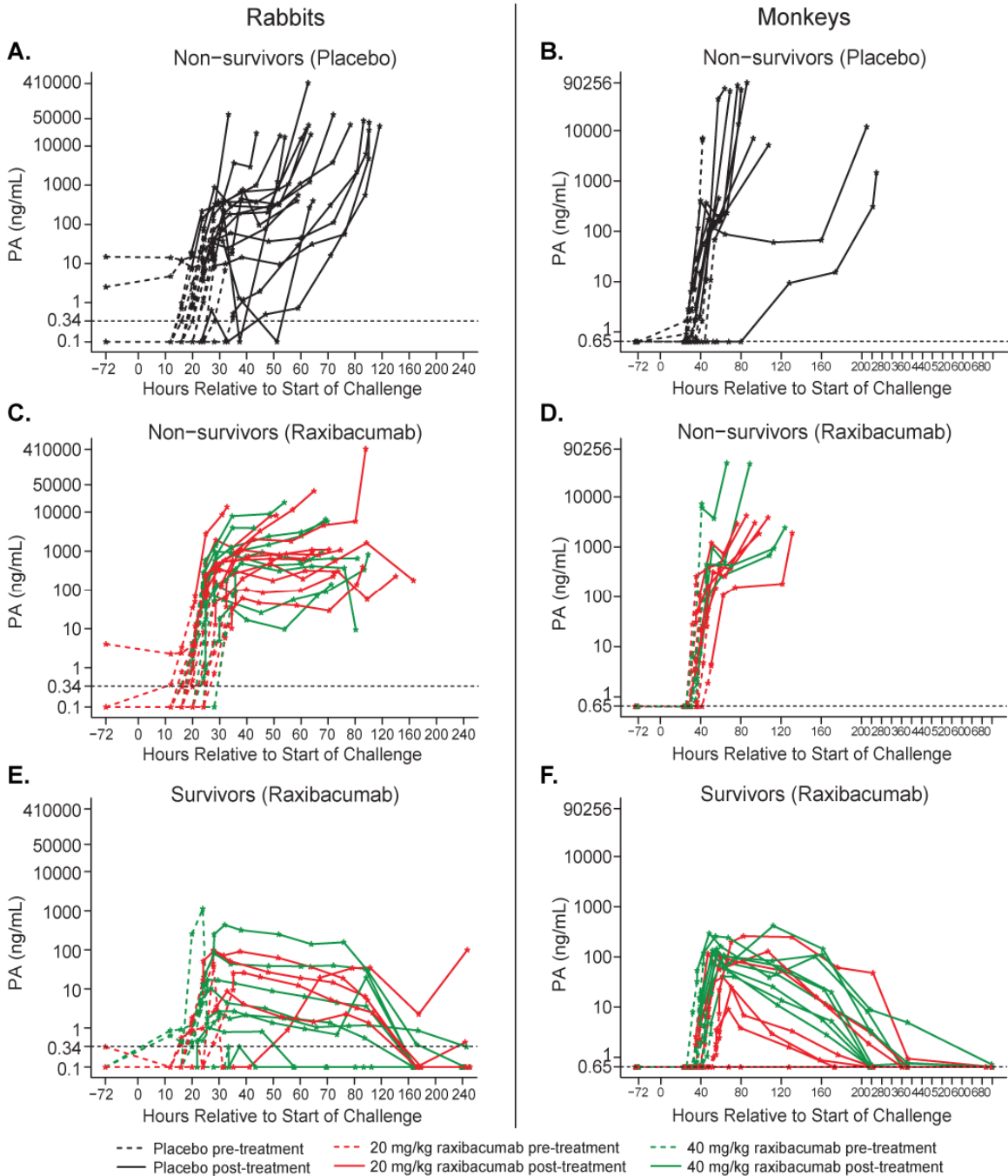
6. Inhalational challenge exposure data

Study Objective	Species	# Subjects by Arm	Anthrax challenge (LD ₅₀)
Characterization	NZW Rabbits	8	178 ± 57
Characterization	Cynomolgus monkeys	8	260 ± 108
Pivotal efficacy; therapeutic treatment	NZW Rabbits	Placebo: 17	222 ± 31
		20 mg/kg: 18	230 ± 37
		40 mg/kg: 18	233 ± 54
Confirmatory efficacy, therapeutic treatment	Cynomolgus monkeys	Placebo: 12	198 ± 44
		20 mg/kg: 14	199 ± 52
		40 mg/kg: 14	157 ± 32
Pre-exposure prophylaxis	NZW rabbits	Placebo	175 ± 95
		1 mg/kg raxi	219 ± 91
		5 mg/kg raxi	197 ± 100
		10 mg/kg raxi	181 ± 64
		20 mg/kg raxi	201 ± 48
		40 mg/kg raxi	243 ± 129
Pre-exposure prophylaxis	Cynomolgus monkeys	Placebo	180 ± 72
		10 mg/kg raxi	187 ± 61
		20 mg/kg raxi	177 ± 79
		40 mg/kg raxi	194 ± 72
Anthrax spore rechallenge	Cynomolgus monkeys	Naïve	154 ± 62
		Survivors	179 ± 76

6. Rabbit and monkey characterization studies: individual animal data



7. Serum PA levels and association between raxibacumab:PA ratio and survival in rabbits and monkeys. PA levels over time by individual rabbits (left) and monkeys (right) are provided for placebo-treated non-survivors (Panels A-B), raxibacumab-treated non-survivors (Panel C-D), and raxibacumab-treated survivors (Panels E-F).



8. Necropsy, histopathology and tissue bacteremia - Gross necropsy was performed on all animals that were found dead or were euthanized according. Sections of target tissues including but not limited to brain/meninges, lungs, liver, spleen, kidney and mediastinal lymph nodes as well as all gross lesions will be preserved in 10% neutral buffered formalin. Histopathology will be performed by a board-certified Veterinary Pathologist, to confirm death or illness due to anthrax. A representative sample of spleen, liver, and mediastinal lymph node may be collected during gross necropsy, homogenized, and

plated on blood agar plates for qualitative assessment of bacterial presence in the target tissues.

Summary of Selected Microscopic Findings with Average Severity (Monkeys Found Dead or Euthanized in Moribund Condition)

Group*		Males			Females		
		placebo	20mg/kg	40mg/kg	placebo	20mg/kg	40mg/kg
Brain	n	6	4	2	6	3	3
	Bacteria	6	3	2	6	2	3
	(average severity)	(1.2)	(2.3)	(2.0)	(1.2)	(2.3)	(2.0)
	Hemorrhage	1	3	1	1	3	2
	(average severity)	(0.2)	(2.3)	(2.0)	(0.5)	(3.0)	(3.0)
Kidney	n	6	4	2	6	3	3
	Bacteria	6	3	2	5	0	1
	(average severity)	(2.0)	(0.8)	(1.0)	(1.5)	(0.0)	(0.3)
	Liver	6	4	2	6	3	3
	Bacteria	6	2	1	5	1	2
	(average severity)	(1.7)	(0.5)	(0.5)	(1.7)	(0.3)	(0.7)
Lung	n	2	4	6	3	3	6
	Bacteria	1	2	6	3	1	5
	(average severity)	(1.5)	(0.5)	(2.2)	(1.0)	(0.3)	(2.2)
	Hemorrhage	0	1	1	0	0	2
	(average severity)	(0.0)	(0.3)	(0.2)	(0.0)	(0.0)	(0.7)
Mediastinal lymph node	n	6	4	2	5	3	3
	Bacteria	6	4	1	5	1	1
	(average severity)	(1.7)	(1.3)	(0.5)	(0.8)	(0.3)	(0.3)
	Hemorrhage	5	1	1	2	0	1
	(average severity)	(1.5)	(0.3)	(0.5)	(0.5)	(0.0)	(0.3)
Spleen	n	6	4	2	6	3	3
	Bacteria	5	3	1	5	1	2
	(average severity)	(3.2)	(1.3)	(2.0)	(2.8)	(0.3)	(1.3)
	Lymphoid depletion/necrosis	5	2	2	6	3	3
	(average severity)	(1.7)	(0.8)	(2.0)	(2.2)	(1.0)	(1.0)

Summary of Incidence of Selected Microscopic Findings with Average Severity (Rabbits Found Dead or Euthanized in Moribund Condition)

Group*		Males			Females		
		placebo	20mg/kg	40mg/kg	placebo	20mg/kg	40mg/kg
Brain	n	9	8	4	8	4	6
	Bacteria	2	7	4	1	2	3
	(average severity)	(0.3)	(2.6)	(1.8)	(0.3)	(1.3)	(1.7)
	Hemorrhage	3	7	4	1	3	3
	(average severity)	(0.6)	(2.0)	(2.5)	(0.4)	(1.8)	(1.5)
Kidney	n	9	8	4	8	4	6
	Bacteria	8	2	0	8	2	2
	(average severity)	(1.8)	(0.3)	(0.0)	(2.3)	(1.3)	(0.8)
Bronchial Lymphnode	n	9	8	4	8	4	6
	Bacteria	8	1	1	8	2	3
	(average severity)	(2.8)	(0.1)	(0.3)	(3.4)	(2.0)	(1.3)

Lung	n	9	8	4	8	4	6
Bacteria (average severity)		7 (2.6)	0 (0.0)	0 (0.0)	8 (3.0)	2 (0.5)	1 (0.2)
Hemorrhage (average severity)		0 (0.0)	1 (0.1)	0 (0.0)	5 (1.3)	0 (0.0)	0 (0.0)
Mediastinal lymph node	n	9	8	4	8	4	6
Bacteria (average severity)		7 (3.0)	1 (0.1)	0 (0.0)	7 (3.3)	2 (2.0)	4 (1.7)
Hemorrhage (average severity)		8 (2.4)	1 (0.1)	2 (1.8)	7 (1.4)	0 (0.0)	3 (1.7)
Spleen	n	9	8	4	8	4	6
Bacteria (average severity)		7 (2.8)	0 (0.0)	0 (0.0)	8 (3.0)	2 (1.0)	1 (0.3)
Lymphoid depletion/necrosis (average severity)		6 (1.7)	0 (0.0)	0 (0.0)	2 (0.5)	2 (0.8)	1 (0.5)

8. Summary of Human studies:

Clinical Study	Number of Subjects Exposed						
	Placebo			Raxibacumab			
	Single Dose (< 40 mg/kg raxibacumab)	Single Dose (40 mg/kg raxibacumab equiv)	Double Dose (40 mg/kg raxibacumab equiv)	Single Dose < 40 mg/kg	Single Dose 40 mg/kg	Double Dose 40 mg/kg 14 days apart	Double Dose 40 mg/kg > 4 months apart
PAM-NH-01	23	2	-	73	7	-	-
HGS1021-C1064	-	-	-	-	86 ¹	-	-
HGS1021-C1069	-	-	-	-	-	-	20
HGS1021-C1063	-	74	6 ⁴	-	217 ²	23 ^{2,3}	-
Total exposed by dose	23	76	6	73	310	23	20
Total subjects exposed by study agent	105			406 ⁵			
¹ Three subjects received a partial dose ² One subject received a partial dose ³ One subject randomized to the double-dose group received only their first dose of raxibacumab and is included with the 217 single-dose subjects ⁴ Two subjects randomized to the double-dose group received only their first dose of placebo and are included with the 74 single-dose subjects ⁵ The 20 subjects from Study HGS1021-1069 are not included in the total subjects exposed by study agent, as they received their first dose of raxibacumab in Study HGS1021-C1064.							

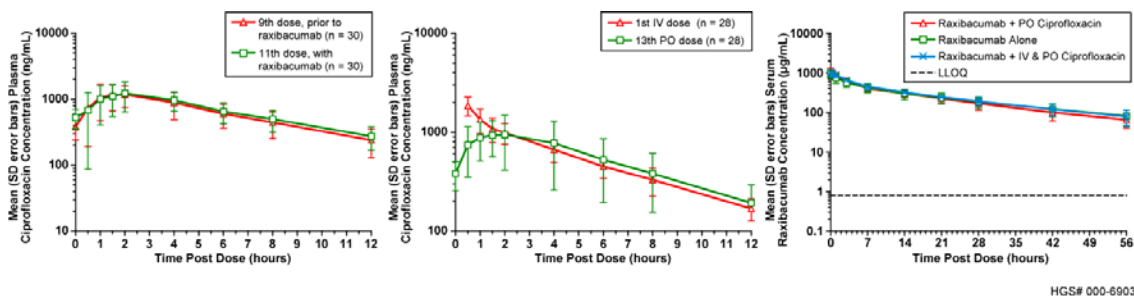
A total of 310+23 = 333 subjects received raxibacumab alone or in combination with ciprofloxacin.

PAM-NH-01: Phase 1, placebo controlled dose escalation study.

HGS1021-1063: Randomized, placebo controlled, raxibacumab [single or double dose]

HGS1021-C1069 was an open-label study to evaluate the immunogenicity and safety of repeat-dose raxibacumab 40 mg/kg IV in healthy subjects who had received a dose of raxibacumab \geq 4 months prior to this study. In total, 20 subjects were treated per protocol.

HGS1021-C1064 was an open-label study to evaluate the pharmacokinetics and safety of 40 mg/kg IV raxibacumab administered in combination with ciprofloxacin, conducted at 3 US sites from January to August 2007. Treatment Group 1 received PO ciprofloxacin (500 mg Q12h x 13 doses), with a single raxibacumab dose IV on Day 5. Group 2 received a single raxibacumab dose IV on Day 0. Group 3 received two IV ciprofloxacin (400 mg) doses given 12 h apart on Day 0, and at 12 hours after the 2nd IV dose, PO ciprofloxacin (500 mg Q12h) for a total of 13 doses. In total, 88 subjects were treated per protocol. Raxibacumab was well tolerated when administered with ciprofloxacin. The PK of either drug remains unaltered when co-administered.



HGS1021-C1069 was an open-label study to evaluate the immunogenicity and safety of repeat-dose raxibacumab 40 mg/kg IV in healthy subjects who had received a dose of raxibacumab \geq 4 months prior to this study. In total, 20 subjects were treated per protocol.

Supplemental Safety Information: Table 1

Related adverse events included the following: placebo group – leukocytosis, neutropenia, tachycardia, abdominal pain, diarrhea, nausea, increased energy, pharyngitis, upper respiratory tract infection, arthralgia, muscular weakness, myalgia, pain in extremity, dizziness, headache, tension headache, cough, hyperhidrosis, urticaria, pallor, superficial thrombophlebitis; raxibacumab group – anemia, leucopenia, neutropenia, vertigo, diarrhea, nausea, stomach discomfort, fatigue, infusion site pain, infusion site phlebitis, malaise, non-cardiac chest pain, peripheral edema, cholecystitis, nasopharyngitis, rhinitis, procedural pain, increased blood amylase, increased blood pressure, arthralgia, musculoskeletal pain, myalgia, pain in extremity, clonus, dizziness, headache, lethargy, paresthesia, somnolence, anxiety, rash (erythematous, macular, popular, pruritic), skin exfoliation, skin hyperpigmentation, urticaria, flushing.

Grade 2 or higher adverse events included the following: placebo group – leukocytosis, neutropenia, eustacian tube dysfunction, abdominal pain, lower abdominal pain, irritable bowel syndrome, vomiting, vessel puncture site pain, gastroenteritis, skin infection, injury, procedural pain, increased blood glucose, headache, tension headache, glycosuria, cough, dyspnea, hyperhidrosis, pallor; raxibacumab group – leukopenia, lymphadenopathy, palpitations, tympanic membrane perforation, abdominal pain, colitis, diarrhea, nausea, infusion site phlebitis, non-cardiac chest pain, cholecystitis, gastroenteritis, influenza, nasopharyngitis, otitis externa, rhinitis, sinusitis, tooth abscess, upper respiratory tract infection, back injury, clavicle fracture, contusion, joint sprain,

muscle strain, procedural pain, increased blood amylase, increased creatine phosphokinase, prolonged prothrombin time, arthralgia, joint stiffness, muscle spasms, tendonitis, dizziness, headache, migraine, somnolence, syncope vasovagal, schizophrenia, dysmenorrhea, ovarian cyst ruptured, cough, dyspnea, sinus congestion, throat irritation, pruritis, rash, skin exfoliation, superficial phlebitis.

9. Tables to support K-M time to event plots:

Figure 1A: Number of Animal at Risk

Day	Placebo	1mg/kg	5mg/kg	10mg/kg	20mg/kg	40mg/kg
0	12	12	12	12	12	12
2	11	11	12	12	12	12
4	0	5	12	12	12	12
6	0	0	9	12	11	12
8	0	0	5	10	10	12
10	0	0	5	10	10	12
12	0	0	5	10	10	12
14	0	0	5	10	10	12

Figure 1B: Number of Animal at Risk

Day	Placebo	10mg/kg	20mg/kg	40mg/kg
0	10	10	10	10
2	10	10	10	10
4	8	10	10	10
6	2	8	8	9
8	0	6	7	9
10	0	6	7	9
12	0	6	7	9
14	0	6	7	9
16	0	6	7	9
18	0	6	7	9
20	0	6	7	9
22	0	6	7	9
24	0	6	7	9
26	0	6	7	9
28	0	6	7	9

Figure 2 - Rabbit

Day	Number of animal at risk
0	8
1	8
2	8
3	6
4	3
5	1
6	1

7	1
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Figure 2 - Monkey

Day	Number of animal at risk
0	8
2	8
4	6
6	3
8	2
10	2
12	2
14	2
16	2
18	2
20	2
21	2

Figure 3A: Number of Animal at Risk

Day	Placebo	20mg/kg	40mg/kg
0	17	18	18
2	15	16	16
4	3	7	9
6	0	6	8
8	0	5	8
10	0	5	8
12	0	5	8
14	0	5	8

Figure

3B: Number of Animal at Risk

Day	Placebo	20mg/kg	40mg/kg
0	12	14	14
2	11	14	14
4	3	10	11
6	2	7	9
8	2	7	9
10	1	7	9
12	0	7	9
14	0	7	9
16	0	7	9
18	0	7	9
20	0	7	9
22	0	7	9
24	0	7	9
26	0	7	9
28	0	7	9

10. Statistical analysis: . The results were to be considered statistically significant if at least 1 of the pairwise comparisons between raxibacumab and placebo achieved a p value < 0.025 , or both pairwise comparisons between raxibacumab and placebo achieved a p value < 0.05 .