Supplement to *Pre- and Postlicensure Animal Efficacy Studies Comparing Anthrax Antitoxins*

By Slay RM et al.

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Supplementary Text. Animal Efficacy Studies Comparing Unlicensed Anthrax Antitoxins

INTRODUCTION

Two anti-protective antigen (PA) monoclonal antibody (mAb) antitoxins and a polyclonal antibody (pAb) were developed by Emergent Biosolutions and Pharmathene, Inc.: AVP-21D9 (Thravixa [mAb]), MDX-1303 (Valortim® [mAb]), and AIGIV, (Anthravig [pAb]) (See Table 1, main manuscript) These 3 non-FDA-approved anti-PA antitoxins were evaluated for therapeutic efficacy in New Zealand White rabbits exposed to a lethal aerosol dose of *Bacillus anthracis* Ames spores at Battelle Biomedical Research Center (BBRC) and the University of Texas Medical Branch (UTMB).

The BBRC study evaluated AIGIV-Anthravig, AVP-21D9 and MDX-1303 concurrently with the FDA-approved antitoxin raxibacumab. The UTMB study evaluated MDX-1303 concurrently with the FDA-approved antitoxin obiltoxaximab. The studies described in this supplement addresses the therapeutic efficacy (ie, survival and morbidity) of AIGIV-Anthravig, AVP-21DP, and MDX-1303 and is complementary to the manuscript entitled *Pre- and Postlicensure Animal Efficacy Studies Comparing Anthrax Antitoxins*.

MATERIALS AND METHODS

Test System

For phase 1 at BBRC, 88 specific pathogen-free New Zealand White (NZW) rabbits (*Oryctolagus cuniculus*; 50% male, 50% female) weighing 2.5 to 4.5 kg were procured without regard to age from Covance (Denver, PA). All had pre-placed, surgically implanted vascular access port (VAPs) and temperature and activity (TA) transmitters. The animals were split into 4 test groups and an untreated control group with 16 animals each; there were 8 extra animals. Three unapproved products were evaluated against placebo concurrently with the approved antitoxin described in the main text: AIGIV-Anthravig, AVP-21D9, and MDX-1303. Only the 48 animals used to evaluate these 3 unapproved antitoxins are discussed here.

For phase 2 at UTMB, 26 NZW rabbits (50% male, 50% female) with pre-implanted VAPS were purchased from Covance (Denver, PA). The animals were split into 2 test groups with 10 rabbits each and an untreated control group of 6. One unapproved product, MDX-1303, was evaluated against a placebo alongside the approved antitoxin described in the main text. Only the 10 rabbits used to evaluate the unapproved antitoxin are discussed here.

The patency of each VAP was maintained to ensure sterility was preserved for accurate blood draws and for administration of antitoxins and placebo. Animals in each study were randomly assigned to test or control groups, and the identity of each animal was confirmed.

With only the rabbit's muzzle placed in the exposure chamber, animals were exposed to a lethal challenge of *B. anthracis* Ames spores and followed for 28 days. Each animal was observed during the 28-day period for clinical signs of infection. Any animal that appeared to be succumbing to the disease was euthanized. Animals alive at the end of 28 days were considered survivors and were sacrificed.

Test Articles

A common therapeutic dose of each of the mAbs (20mg/kg) and an optimal pAb dose (14.2mg anti-PA/kg) were evaluated. Each antitoxin was administered at onset of antigenemia, which was determined using a PA-electrochemiluminescent assay (PA-ECL) to detect the presence of protective antigen in the blood of exposed rabbits.

BBRC. At positive antigenemia, test groups received the indicated dose of AVP-21D9 or MDX-1303, delivered as a single bolus injection through the VAP at a volume based on the body weight of each animal measured on day -1. Because the 3.6mL/kg was administered to each designated animal in a volume too large for a single bolus injection, both AIGIV and the placebo were delivered as slow IV infusions. Administration of the antitoxins was semi-blinded due to this difference in route. Although the identities of the antitoxins and control were hidden from administrators, those delivered via single bolus injection (both mAbs) were distinguishable from those delivered as a slow infusion (pAb and placebo). Samples of each test article preparation were analyzed to confirm the dose concentration. As validated assays for the 2 investigational mAbs were not available and an available ELISA assay proved inaccurate, a toxin neutralization assay was used to approximate dose concentrations of each antitoxin. This assay was based on the assumption that the neutralization factor 50 (NF50) measurements would correlate with anti-PA IgG ELISA determinations. This method estimated there was at least 81% of the expected neutralizing activity.

UTMB. MDX-1303 was provided as a stock solution. Prior to dosing, animals were weighed and the stock mAb solution was diluted in 0.9% sodium chloride so that a constant dose volume of 2 mL, containing the dose of 20mg/kg, was delivered via the VAP as a single bolus injection. For the UTMB study, the treatment groups were not blinded and dose concentrations were not confirmed.

Aerosol Challenge

BBRC. Given the known starting spore concentration of a suspension, an aerosol concentration was calculated to achieve a targeted inhaled dose of 200 lethal dose 50 (LD_{50}) (ie, 200 x 1 LD_{50} [1.05×10^5 spores] = 2×10^7 *B. anthracis* Ames spores). The total accumulated tidal volume (TATV) of each animal was determined to estimate the aerosol volume needed to achieve the target dose of spores based on the spore concentration. Plethysmography was used to measure the volume an animal was breathing and determine how quickly the TATV could be achieved. See Supplementary Table 1 for average spore challenge doses.

UTMB. A qualified aerosol control platform, comprising a muzzle-only aerosol chamber with computer-controlled humidity, pressure, and air flow, was used for spore challenges. Animals were exposed to the same challenge dose of 200 LD_{50} of *B. anthracis* Ames spores. Plethysmography with elastic bands stretched around the thorax and abdomen was used to measure the accumulated tidal volume (ATV) of individual animals. Aerosol samples were collected continuously so the challenge dose of spores could be confirmed for each animal by serial dilution and plating on blood agar plates. The duration of aerosol delivery was based on aerosol spore concentration and the ATV of each animal. See Supplementary Table 1 for average spore challenge doses.

Pathology

BBRC. Animals that succumbed to challenge and those euthanized after being found moribund or surviving to day 28 underwent a complete gross necropsy. Tissues collected into 10% neutral buffered formalin (NBF) were examined histopathologically by a board-certified pathologist. These included, but were not limited to, brain, lungs, liver, spleen, and mediastinal lymph nodes, along with gross lesions.

UTMB. No pathology was performed for this study.

RESULTS

Although head-to-head studies performed at Battelle and UTMB evaluated both FDA-approved (raxibacumab and obiltoxaximab) and unapproved (AVP-21D9, MDX-1303, and AIGIV-Anthravig) antitoxins, only the data for the 3 nonapproved/investigational antitoxins and placebo are presented here.

Study Metrics

Key design and outcome metrics for the BBRC and UTMB studies used to determine the relative therapeutic potential of the 1 investigational pAb and the 2 investigational mAbs are shown in Supplementary Table 1.

Primary Outcomes: Mortality and Time to Death

BBRC. The key parameter measured during the head-to-head studies was survival following challenge with a lethal dose of *B. anthracis*. As shown in Supplementary Table 1 and Supplementary Figure 1, survival for the animals was 69% in the AVP-21D9 group, 80% in the MDX-1303 group, and 19% in the AIGIV-Anthravig group. All animals that received placebo died within 6 days post challenge.

UTMB. In the UTMB study, as shown in Supplementary Table 1 and Supplementary Figure 2, survival for the animals in the MDX-1303 (20mg/kg) group was 80% compared to 0% in the placebo group.

Statistical analysis. The antitoxin mortality data derive from 2 separate studies with slightly different parameters. Testing for statistical significance between test groups was performed using a pairwise log-rank test adjusted for multiple comparisons by using a Bonferroni correction within, but not between, the two studies. In the BBRC study, both the MDX-1303 and the AVP-21D9 test arms were different from the placebo group ($P < 0.001$ for both) but not from each other ($P = 1.00$). Although survival with the AIGIV-Anthravig test arm was not different from placebo ($P = 0.2258$), a significant delay in time-to-death was observed. In the UTMB study, the MDX-1303 test group was different from the placebo group ($P = 0.0025224$).

Secondary Outcomes: Pathology and Histopathology

BBRC. Histopathology of the challenged rabbits was examined. The lesions observed in animals that were either found dead or humanely euthanized due to morbidity were consistent with anthrax as a cause of death. Also, the microscopic findings were also consistent with the histopathology of anthrax. [1] Average severity scores for microscopic findings are shown in Supplementary Tables 2 and 3.

For male and female rabbits treated with AVP-21D9, MDX-1303, or AIGIV-Anthravig that underwent unscheduled termination or were found dead, microscopic findings were almost entirely in the minimal to mild range. A few instances of some microscopic lesions in the moderate range were observed in 2 male animals in the MDX-1303-treated group (ie, mediastinal lymph node tissue showing moderate inflammation and necrosis). In female rabbits from the AVP-21D9 test group, only a single animal was observed with moderate degeneration/necrosis of the liver. Remarkably, no male and female rabbits treated with AVP-21D9 showed lesions in brain tissue.

CONCLUSIONS

One unapproved anti-PA pAb, AIGIV-Anthravig, and 2 unapproved mAbs, AVP-21D9 and MDX-1303, were evaluated in head-to-head studies at BBRC and UTMB. NZW rabbits were treated with 14.2mg/kg of the pAb or 20mg/kg of a mAb at the onset of antigenemia following exposure to a lethal *B*. *anthracis* Ames spore challenge dose. The relative therapeutic value of MDX-1303 was determined at both BBRC and UTMB, and the rate of survival from both studies was 80%. AVP-21D9 and AIGIV-Anthravig were evaluated only at BBRC, and the rate of survival of these antitoxins was 69% and 19%, respectively. For both mAbs, the improved survival observed in the test groups compared to control groups was statistically significant ($P < 0.05$). The rate of survival achieved with the pAb was not different from that achieved with placebo. In the BBRC study, the survival rates between the 3 test groups (AVP-21D9, MDX-1303, and AIGIV-Anthravig) did not differ from one another. Finally, results from the pathological analyses suggest no remarkable difference in the pathology observed in animals that succumbed to the infection following treatment with any antitoxin.

Observed lesions were generally mild to minimal and consistent with anthrax. Surviving animals exhibited some indicators of chronic inflammation consistent with a previous bacterial infection.

Evaluations of the 3 anti-PA antitoxins tested for their therapeutic benefit against inhalation anthrax, AVP-21D9, MDX-1303, and AIGIV, led to the following conclusions: survival rates for animals treated with AVP-21D9 and MDX-1303 and time-to-death for animals treated with any of the 3 antitoxins were increased when compared to animals treated with placebo.

References for Supplementary Text

1) Twenhafel, N.A. 2010. Pathology of inhalation anthrax animal models. Veterinary Pathology, 47(5), 819-830.

Supplementary Table 1. Study Metrics for the Evaluation of AVP-21D9, MDX-1303, and AIGIV-Anthravig in Rabbits Exposed to a Lethal Challenge of *B. anthracis* Ames Spores

Site	Arm	n	% Male	Mean Weight, kg[a] (min-max)	Mean Spore challenge, LD_{50}	Start trigger, Hours Postchallenge	Antitoxin		Nonsurvivors Mean Time to Death, h (min-max)	Survival n (%)
							Dose	Route		
BBRC	AIGIV-Anthravig	16[c]	50	3.3 (2.8-3.7)	187	29.9[b]	14.2 mg/kg (3.6 mL/kg)	Slow IV Infusion	134 (62-190)	3 (19)
BBRC	MDX-1303	15[d]	50	3.4 (3.0-3.8)	175	29.4[b]	20 mg/kg (2.5 mL/kg)	Single IV bolus injection/VAP	96 (90-106)	12 (80)
BBRC	AVP-212D9	16	50	3.3 (2.9-3.7)	173	27.8[b]	20 mg/kg (2.5 mL/kg)	Single IV bolus injection/VAP	92 (46-219)	11 (69)
BBRC	Placebo[g]	16	50	3.3 (3.0-3.8)	193	29.2[b]	3.6 mL/kg	Slow IV Infusion	80 (56-121)	0 (0)
UTMB	MDX-1303	10	50	3.4(3.0-3.9)	245	N/D[f]	20 mg/kg (2.0 ml/kg)	Single IV bolus injection/VAP	78(36-120)	8 (80)
UTMB	Placebo[e]	6	50	3.3(2.9-3.5)	291	N/D[f]	20 mg/kg (2.0 ml/kg)	Single IV bolus injection/VAP	70(36-96)	0 (0)

Abbreviations: AIGIV, anthrax immunoglobulin intravenous; BBRC, Battelle Biomedical Research Center; IV, intravenous; LD_{50}, median lethal dose; Max, maximum; Min, minimum; N/D, not done; UTMB, University of Texas Medical Branch; VAP, vascular access port.

[a] Animals weighed on study day -1 (BBRC); day of challenge (UTMB).

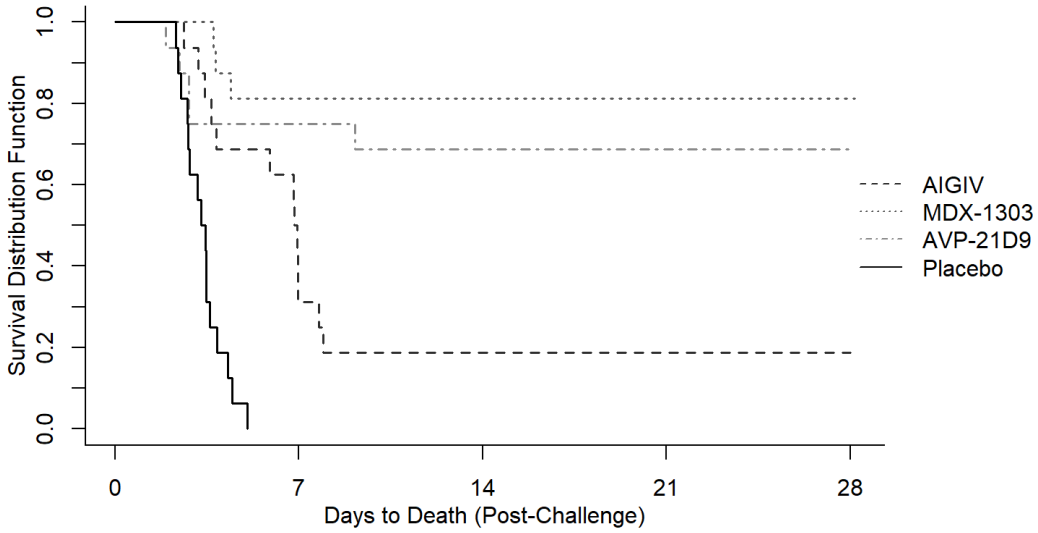
[b] Median time from challenge to abnormal.

[c] During drug administration, slow infusion needle displaced from one animal and full amount of drug was not delivered. Animal succumbed at 88 hours PC. It was included in analyses since impact was judged to be minimal. If excluded from analyses, survival rate is 3 of 15 animals, or, 20%.

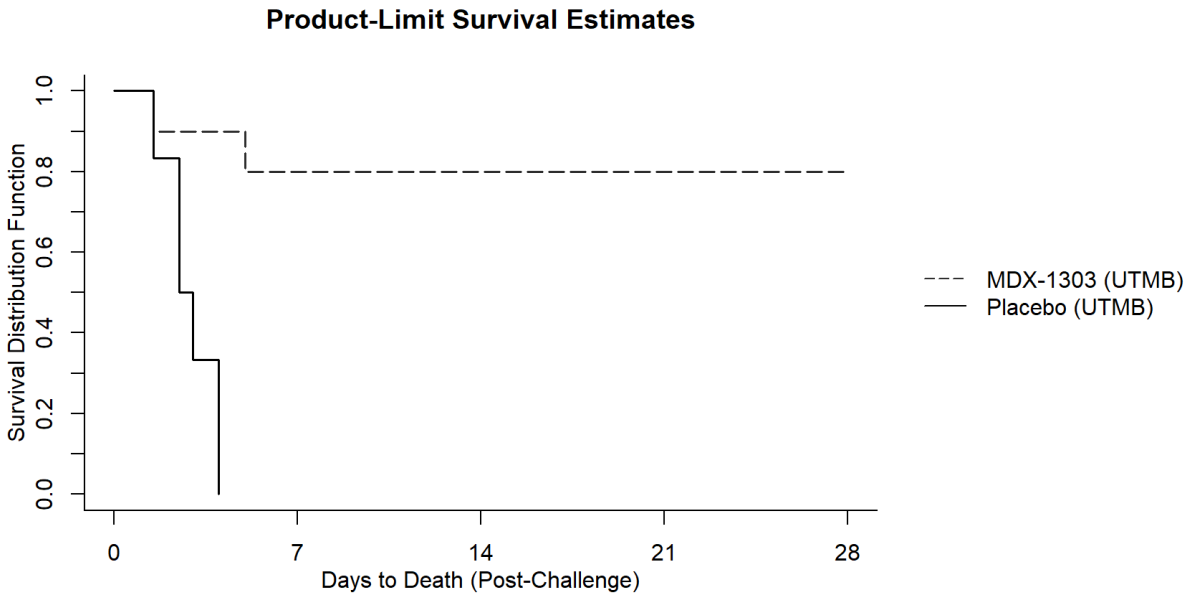
[d] One of 16 animals from the MDX-1303 test group was excluded since it was not positive for blood culture at any time point prior to receiving MDX-1303.

[e] Data for animals given placebo are also presented in Tables 2 and 3 in the main text

[f] MDX-1303 group: PA detected ~19.2 hours post challenge. Placebo group: PA detected ~21 hours post challenge. Treatment initiated 3-4 hours post antigenemia.



Supplementary Figure 1. Kaplan-Meier Plots of AVP-21D9, MDX-1303, and AIGIV-Anthravig for Therapeutic Benefit Against Inhalation Anthrax.



Supplementary Figure 2. Kaplan-Meier Plots of MDX-1303 for Therapeutic Benefit Against Inhalation Anthrax.

Supplementary Table 2. Histopathologic Examination and Scoring of Microscopic Observations in Challenged Male Rabbits that Either Died or Were Euthanized, by Antitoxin

Tissue	Observation	Animals Scored Per Group/Average Severity[a]			
		AIGIV-Anthravig (7 Examined)	MDX-1303 (2 Examined)	AVP-21D9 (3 Examined)	Placebo (8 Examined)
Brain	Bacteria	7/1.4	1/2.0	0/0.0	6/1.5
	Fibrin	2/1.5	0/0.0	0/0.0	1/1.0
	Hemorrhage	2/2.5	1/2.0	0/0.0	2/1.0
	Inflammation	2/1.5	1/2.0	0/0.0	1/1.0
	Necrosis	2/2.0	0/0.0	0/0.0	0/0.0
	Vasculitis	2/1.5	0/0.0	0/0.0	0/0.0
Liver	Bacteria	4/1.0	1/1.0	1/1.0	5/1.4
	Degeneration/Necrosis	3/1.3	2/2.5	4/1.3	4/1.5
	Infiltrate, Cellular	1/1.0	0/0.0	1/1.0	1/1.0
	Inflammation	2/1.0	1/1.0	0/0.0	3/1.0
	Leukocytosis	5/1.0	2/1.0	3/1.0	7/1.0
	Mineralization	ND	1/1.0	0/0.0	0/0.0
Lungs	Bacteria	5/1.4	1/1.0	1/1.0	6/1.8
	Edema	2/1.5	1/2.0	3/1.0	5/1.4
	Fibrin	3/1.7	2/1.0	3/1.0	5/1.0
	Hemorrhage	2/1.5	0/0.0	1/1.0	3/1.0
	Inflammation	5/1.6	2/1.5	4/1.3	7/1.4
	Mineralization	1/1.0	0/0.0	0/0.0	0/0.0
	Thrombosis	1/2.0	1/1.0	0/0.0	0/0.0
	Vasculitis	ND	1/1.0	1/1.0	0/0.0
Lymph Nodes/Mediastinal	Bacteria	5/1.6	1/1.0	2/2.5	6/3.3
	Fibrin	7/1.7	2/3.0	3/2.7	8/2.6
	Hemorrhage	5/2.0	2/2.0	3/1.7	7/1.7
	Infiltrate, Cellular	0/0.0	0/0.0	0/0.0	1/1.0
	Inflammation	4/1.0	2/3.0	3/1.0	7/1.1
	Necrosis	6/1.7	2/3.0	3/2.7	8/3.0
Spleen	Bacteria	5/1.6	1/1.0	3/1.0	6/2.0
	Fibrin	4/2.3	2/2.0	3/2.3	7/1.6
	Hemorrhage	5/1.4	0/0.0	3/1.3	5/1.6
	Necrosis	7/2.1	2/2.0	3/2.3	7/2.0
	Pigment	3/1.3	1/1.0	0/0.0	3/1.0

Abbreviations: AIGIV, anthrax immunoglobulin intravenous; ND, not done.

[a] Severity Score: 1, minimal (least detectable lesion); 2, mild (easily discernable lesion); 3, moderate (change affecting a large area); 4, marked (lesion that approached maximal).

Supplementary Table 3. Histopathologic Examination and Scoring of Microscopic Observations in Challenged Female Rabbits that Either Died or Were Euthanized, by Antitoxin

		Animals Scored Per Group/ Average Severity[a]			
Tissue	Observation	AIGIV-Anthravig (6 Examined)	MDX-1303 (1 Examined)	AVP-21D9 (2 Examined)	Placebo (8 Examined)
Brain	Bacteria	6/1.7	1/1.0	0/0.0	8/1.6
	Fibrin	2/2.0	1/2.0	0/0.0	0/0.0
	Hemorrhage	3/2.0	1/1.0	0/0.0	3/1.7
	Inflammation	2/2.5	1/2.0	0/0.0	1/1.0
	Necrosis	2/2.5	1/2.0	0/0.0	0/0.0
	Vasculitis	2/1.5	1/2.0	0/0.0	0/0.0
Liver	Bacteria	3/1.0	0/0.0	0/0.0	6/1.3
	Degeneration/Necrosis	6/1.5	0/0.0	1/3.0	5/1.4
	Hemorrhage	0/0.0	0/0.0	0/0.0	1/1.0
	Inflammation	3/1.0	1/1.0	1/1.0	2/1.0
	Leukocytosis	6/1.0	0/0.0	2/1.0	7/1.0
Lungs	Bacteria	4/1,8	0/0.0	0/0.0	8/2.0
	Edema	4/1,0	1/1.0	1/1.0	3/1.7
	Fibrin	4/1.0	0/0.0	2/1.5	5/1.6
	Hemorrhage	1/1.0	0/0.0	1/1.0	1/2.0
	Inflammation	6/1.5	1/2.0	2/1.0	7/1.3
	Vasculitis	0/0.0	0/0.0	0/0.0	0/0.0
	Thrombosis	2/1.0	0/0.0	0/0.0	2/1.0
Lymph Nodes/Mediastinal	Bacteria	5/2.2	0/0.0	1/2.0	8/3.9
	Fibrin	5/1.8	1/2.0	2/2.5	8/2.5
	Hemorrhage	3/2.0	1/1.0	1/2.0	8/2.1
	Inflammation	2/1.5	1/1.0	2/1.0	7/1.3
	Necrosis	6/2.0	1/2.0	2/2.0	8/3.3
Spleen	Bacteria	6/1.3	0/0.0	1/1.0	8/2.8
	Fibrin	5/1.6	0/0.0	2/2.0	6/2.0
	Hemorrhage	4/1.5	0/0.0	1/1.0	6/1.3
	Necrosis	6/1.7	1/1.0	2/2.0	8/2.4
	Pigment	4/1.5	0/0.0	1/1.0	5/1.2

Abbreviations: AIGIV, anthrax immunoglobulin intravenous.

[a] Severity Score: 1, minimal (least detectable lesion); 2, mild (easily discernable lesion); 3, moderate (change affecting a large area); 4, marked (lesion that approached maximal).