Animal Models for Anthrax Monoclonal Antibodies

Nonclinical Data for Regulatory Decision-Making on the Efficacy of Medical Countermeasures

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Outline

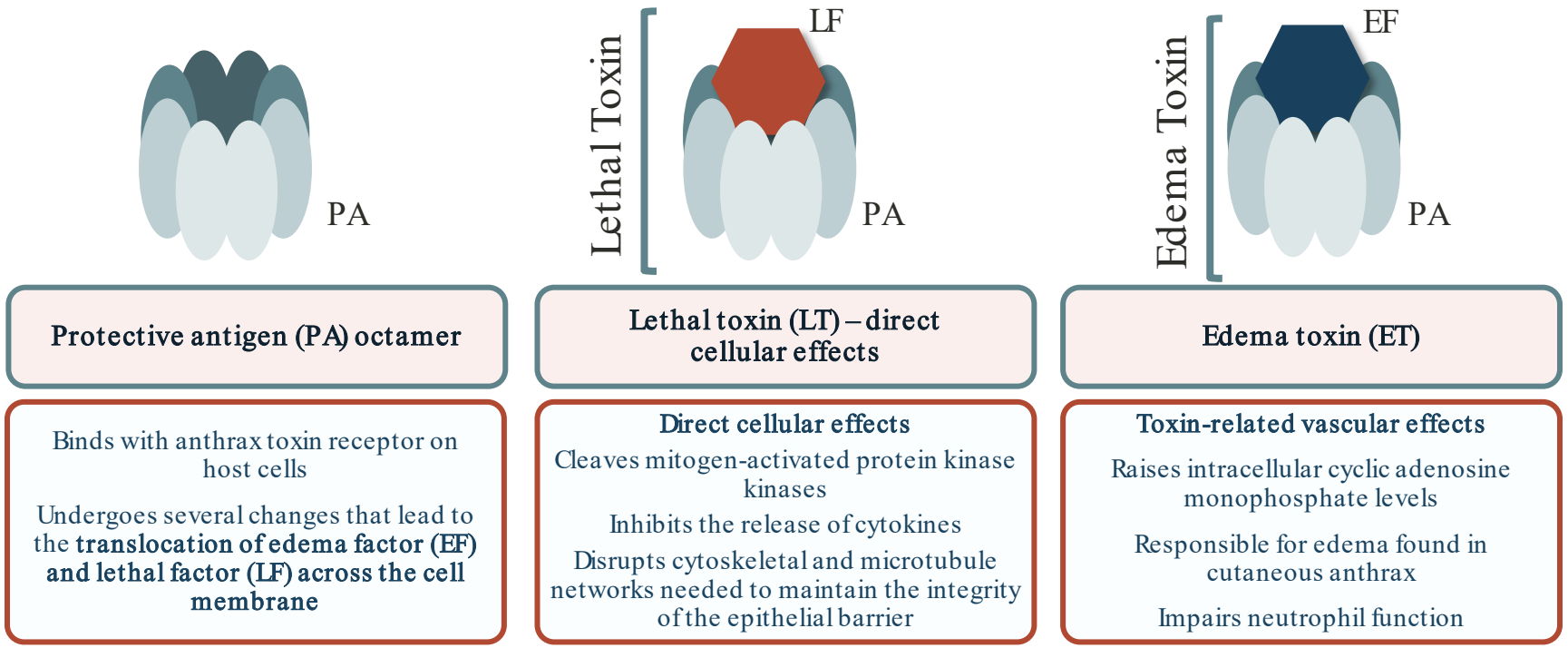
- **Anthrax:**
  - Brief overview of disease
  - Therapeutic targets
  - Medical countermeasures
- **FDA "Animal Rule":**
  - Key principles
  - Approach to demonstration of efficacy
- **Animal Studies:**
  - Animal models of anthrax disease
  - Demonstration of efficacy of therapeutics
  - Bridging animal and human data
  - Combination therapy
- **Summary and Conclusions**

Anthrax Disease Types Based on Route of Exposure to *Bacillus anthracis* spores

	Cutaneous	Gastrointestinal	Inhalational	Intravenous/ Injectional
Occurrence	Endemic areas	Endemic areas	Bioterrorism, biowarfare, sporadic cases (exposure to infected animals or animal products)	Intravenous drug users
Incubation Period	1-17 days	2-5 days	1-6 days (up to 43 reported)	1-10 days
Mortality	<1%	4-50%	85-90%	>30%

Table adapted from Savransky et al. *Current status and trends in prophylaxis and management of anthrax disease.* Pathogens 2020; 9(5): 370.

Anthrax: Tripartite Exotoxin-Mediated Disease



LF and EF have no known activity on the host until they bind to PAand are translocated to the target-cell cytosol.

Figures adapted from Ascenzi et al. *Anthrax toxin: a tripartite lethal combination.* FEBS Lett. 2002; 531(3):384.

Opportunities for Medical Intervention

Pre-exposure prophylaxis (PrEP):

Protect individuals before exposure that may occur in the future

Post-exposure prophylaxis (PEP):

In cases of suspected or confirmed exposure may prevent the development of disease

Therapeutics:

Help reduce morbidity and mortality in symptomatic individuals

- Concurrent vaccination and antibacterial/antitoxin therapy provides optimal clinical outcomes
- Antibiotics provide protection during time required for vaccine to induce protective immune response
- Vaccines provide protection against spores that persist longer than 60-day antibiotic course

Adapted from Bower et al. *CDC Guidelines for the Prevention and Treatment of Anthrax, 2023*. MMWR Recomm Rep. 2023 Nov 17;72(6):1-47.

Role of Antitoxin Therapeutics

- **Advantages:**
  - Anthrax is a toxin-mediated disease, and antimicrobials are ineffective against toxemia
  - Thus, antitoxin therapy may enhance survival by targeting toxin accumulated in the serum
- **Limitations:**
  - Do not have antibacterial activity; therefore, should be used in conjunction with antimicrobials
  - Do not cross the blood-brain barrier; therefore, cannot prevent or treat anthrax meningitis
  - May interfere with vaccine-induced anti-PAantibody generation
- **Approved Products:**
  - Monoclonal: raxibacumab, obiltoxaximab (Anthim®)
  - Polyclonal: anthrax immune globulin intravenous (human) (AIGIV, ANTHRASIL®)

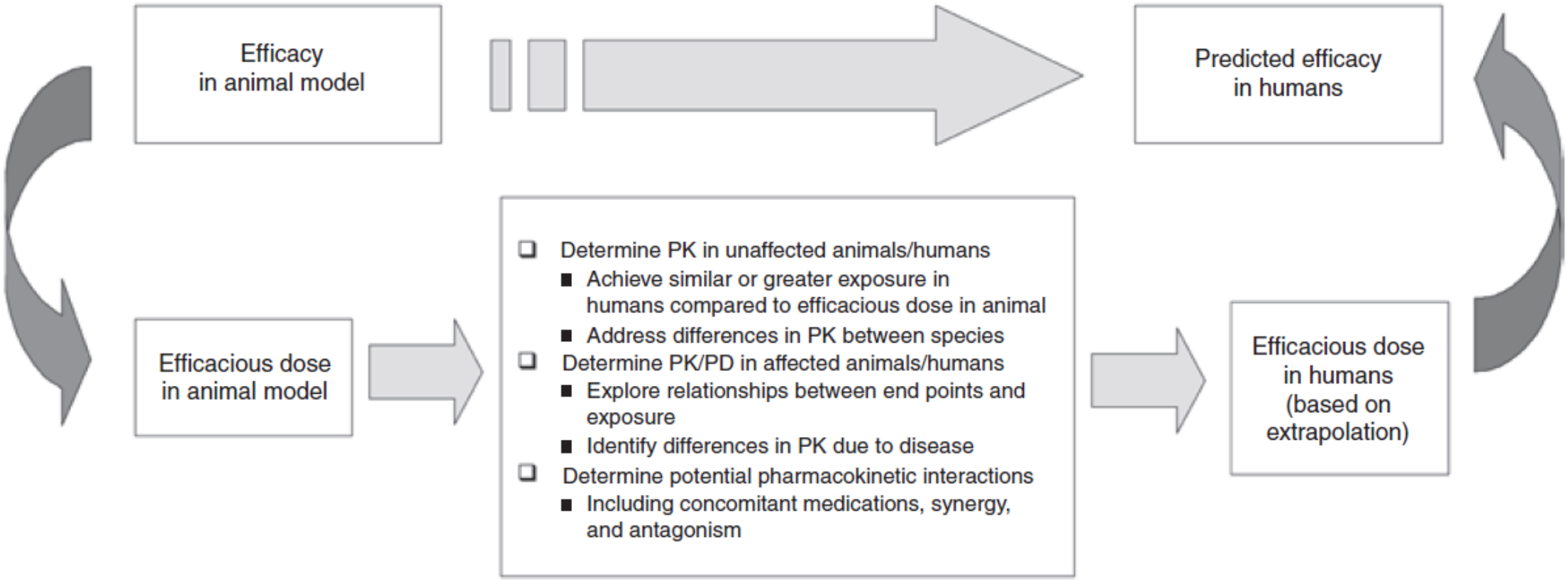
- Huang et al. Antitoxin treatment of inhalation anthrax: a systematic review. *Health Secur.* 2015; 13(6):365-77.
- Malkevich et al. Effect of anthrax immune globulin on response to BioThrax (anthrax vaccine adsorbed) in New Zealand White rabbits. *Antimicrob. Agents Chemother.* 2013; 57(11):5693-6.
- Savransky et al. *Current status and trends in prophylaxis and management of anthrax disease.* Pathogens 2020; 9(5): 370.
- Sweeney et al. Anthrax infection. *Am J Respir Crit Care Med.* 2011; 184(12):1333-41.

U.S. Food and Drug Administration (FDA) Animal Rule

- Applies when "human efficacy studies cannot be conducted because the conduct of such trials is unethical and field trials after an accidental or deliberate exposure are not feasible."
- "FDA will rely on evidence from animal studies to provide substantial evidence of effectiveness only when all of the following four criteria are met:
    1. There is a reasonably well-understood pathophysiological mechanism of the toxicity of the substance and its prevention or substantial reduction by the product;
    2. The effect is demonstrated in more than one animal species expected to react with a response predictive for humans, unless the effect is demonstrated in a single animal species that represents a sufficiently well-characterized animal model for predicting the response in humans;
    3. The animal study endpoint is clearly related to the desired benefit in humans, generally the enhancement of survival or prevention of major morbidity; and
    4. The data or information on the kinetics and pharmacodynamics of the product or other relevant data or information, in animals and humans, allows selection of an effective dose in humans."
- "Clinical trials in healthy humans should evaluate safety and pharmacokinetics data over a range of doses."
- Post-marketing field studies are required "to provide evaluation of safety and clinical benefit… in the event an emergency arises and the drug is used."

US FDA. *Product development under the animal rule – guidance for industry.* 2015. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/product-development-under-animal-rule.

Demonstrating Efficacy of Therapeutics under Animal Rule



Bergman. The animal rule and emerging infections: the role of clinical pharmacology in determining an effective dose. *Clin Pharmacol Ther.* 2009; 86(3):328-31.

Susceptibility of Laboratory Animals to *Bacillus anthracis* Infection

Species	Inhalational LD_{50} (No. of Spores)	Primary Pathophysiological Factors(s)
Mouse	14,500	Bacteremia
Guinea pig	16,650-40,000	Bacteremia, toxemia
Rat	Not determined	Toxemia (resistant to infection)
Hamster	Not determined	Bacteremia
Rabbit	105,000	Toxemia
Cynomolgus macaque	34,000-110,000	Toxemia
Rhesus macaque	30,000-172,000	Toxemia
African green monkey	10,000	Toxemia

Table adapted from Savransky et al. *Current status and trends in prophylaxis and management of anthrax disease.* Pathogens 2020; 9(5): 370.

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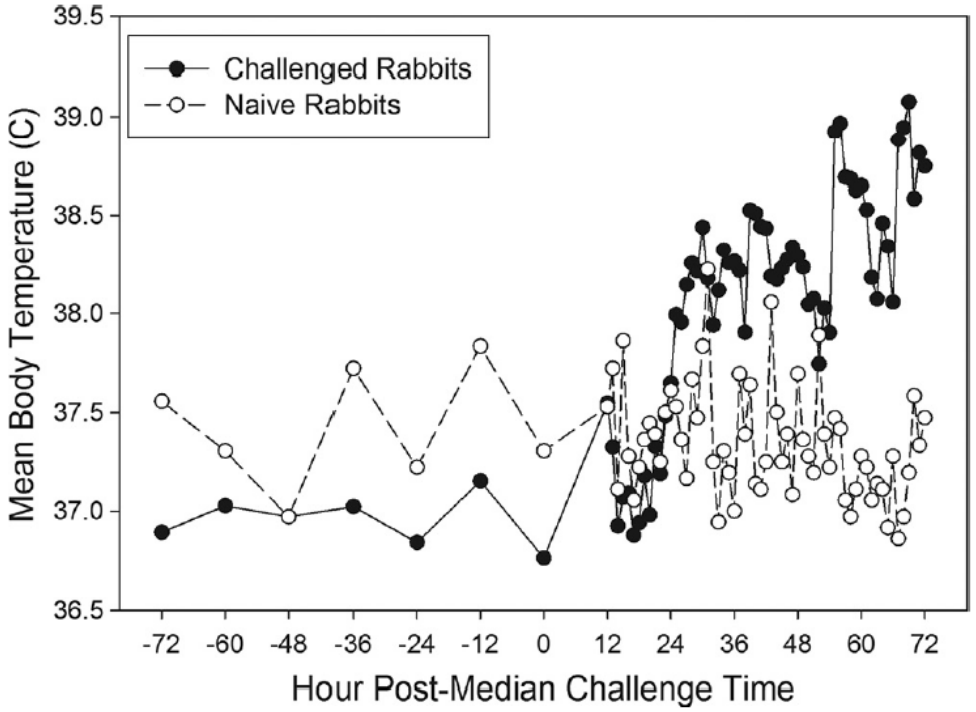
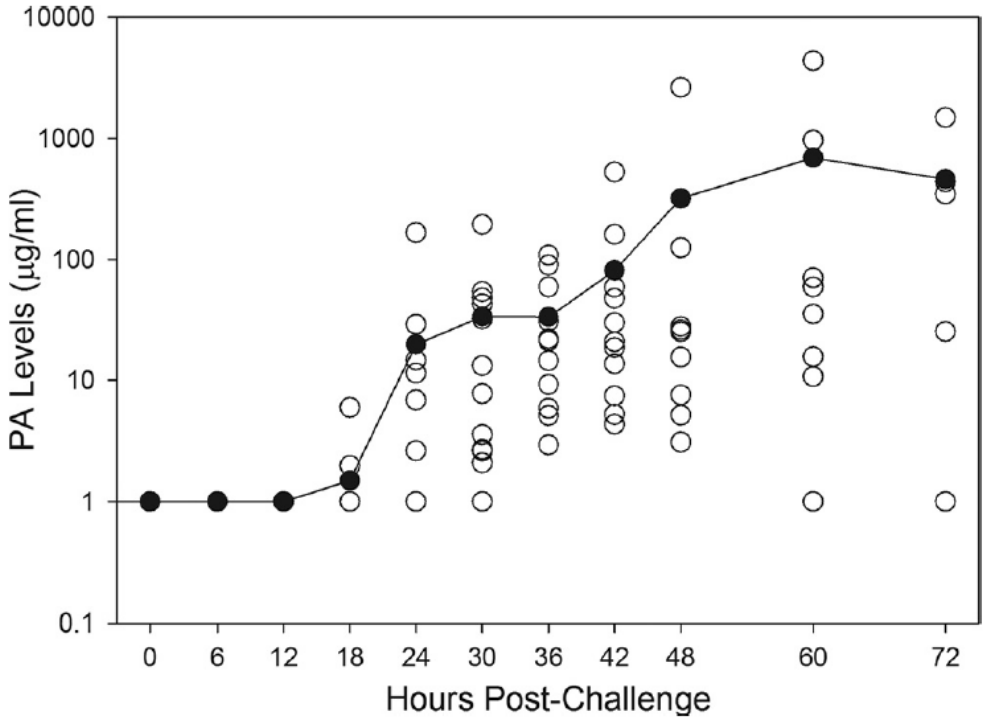
Animal Models for Anthrax Therapeutics: Rabbits





- Significant increase in body temperature correlates with both bacteremia and serum PA.
- Thus, it is a suitable trigger for treatment initiation in rabbit a therapeutic model of inhalational anthrax.

Comer et al. Characterization of a therapeutic model of inhalational anthrax using an increase in body temperature in New Zealand White rabbits as a trigger for treatment. *Clin. Vaccine Immunol* 2012; 19(9):1517-25.

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Animal Models for Anthrax Therapeutics: NHPs

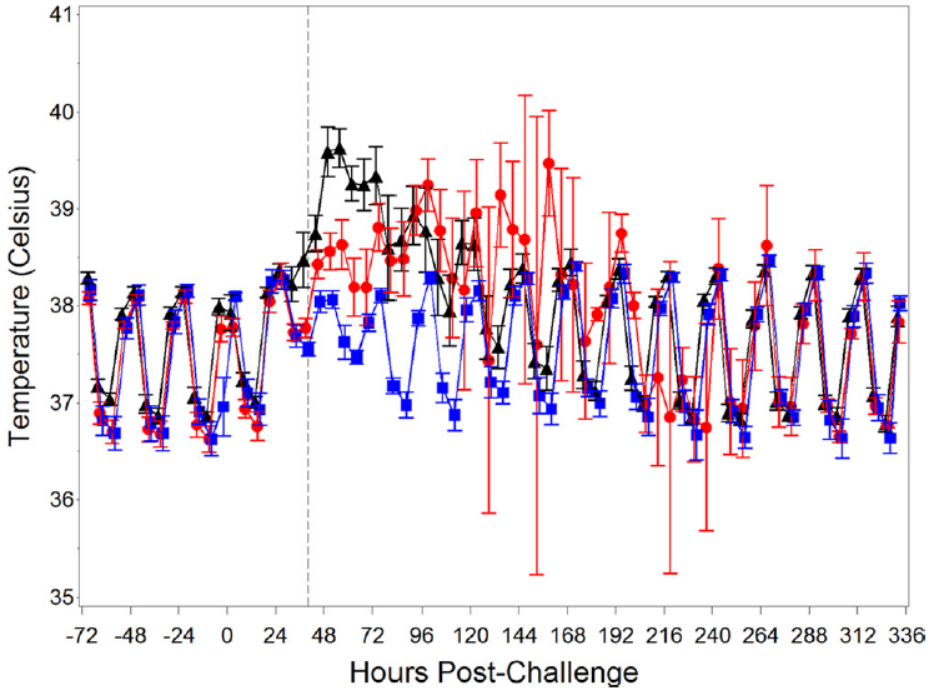


FIG 3 Kinetics of body temperature for challenged and unchallenged animals. The mean body temperatures (± the standard errors of the mean) for *B. anthracis*-challenged and treated (black), *B. anthracis*-challenged and untreated (red), and unchallenged (blue) groups are shown. The dotted line represents the average time to treatment for treated animals.

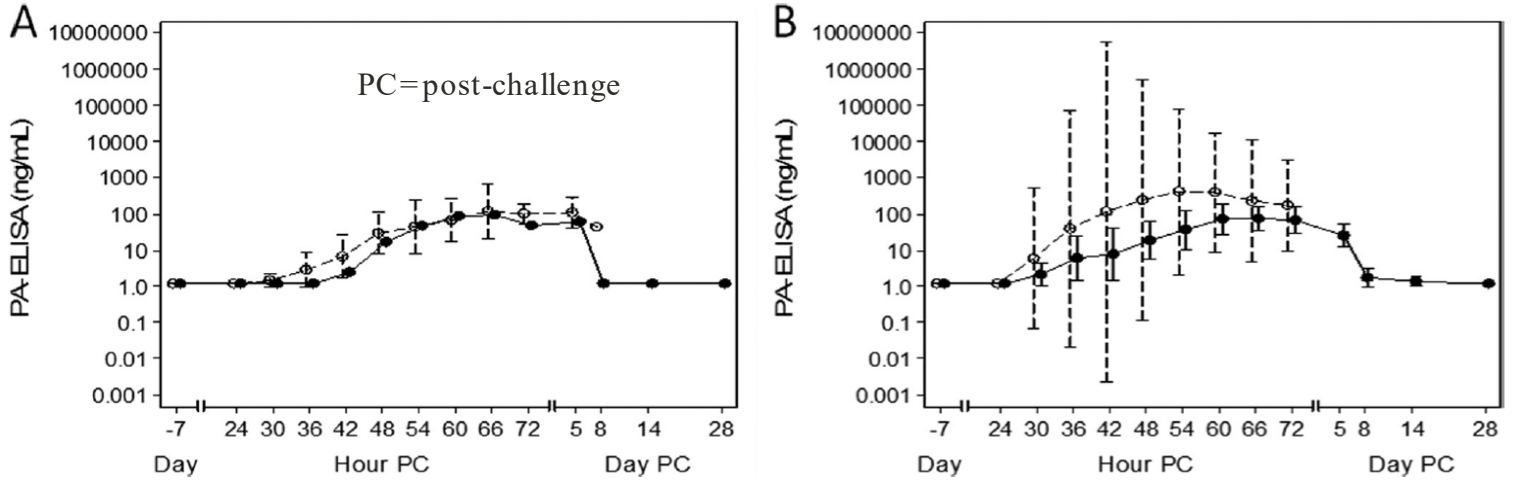
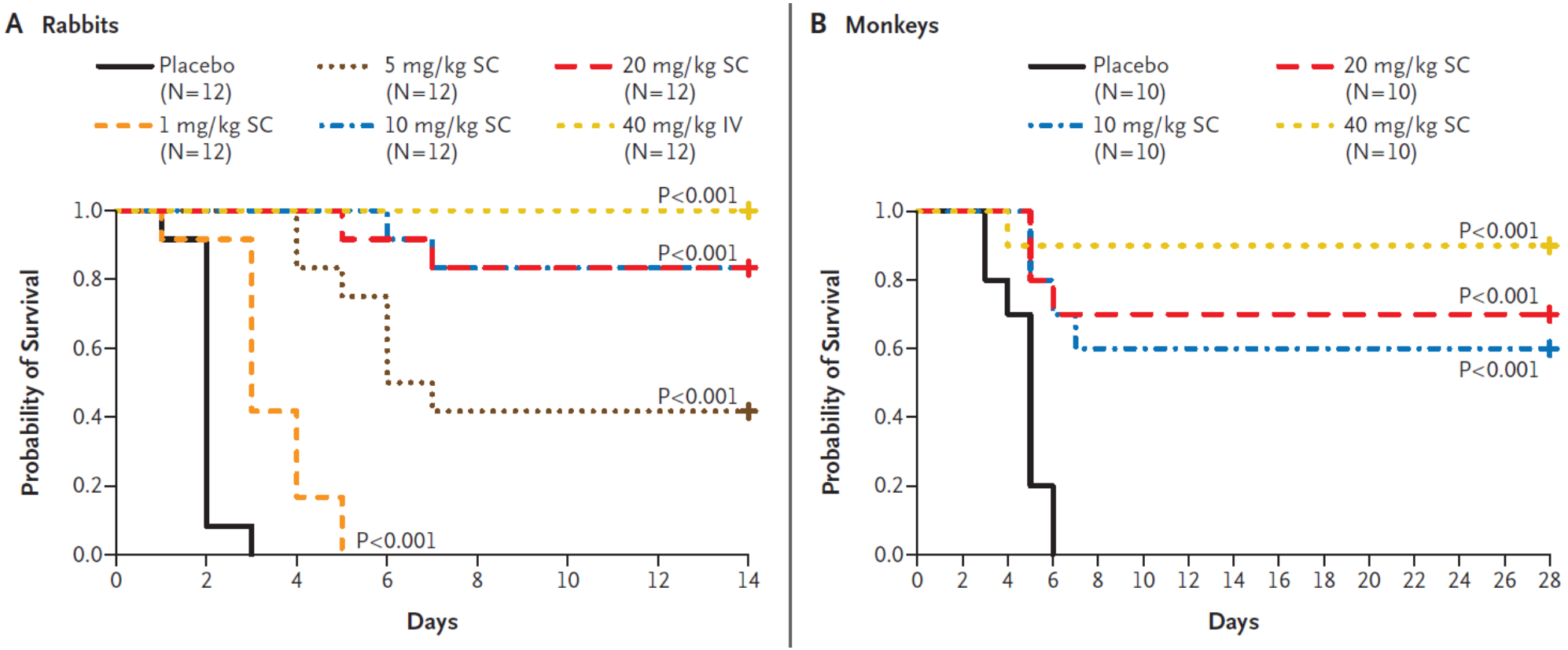


FIG 2 Circulating levels of PA. The circulating PA levels as assessed by ELISA are presented for the untreated animals (open circles, average of nonsurvivors; closed circles, average of survivors) (A) and treated animals (open circles, average of nonsurvivors; closed circles, average of survivors) (B).

- Serum PAdetection, but not increase in body temperature, correlates with bacteremia clinical signs of disease.
- Thus, serum PAis a suitable trigger for treatment initiation in NHP therapeutic model of inhalational anthrax.

Henning et al. Development of an inhalational *Bacillus anthracis* exposure therapeutic model in cynomolgus macaques. *Clin. Vaccine Immunol* 2012; 19(11):1765-75.

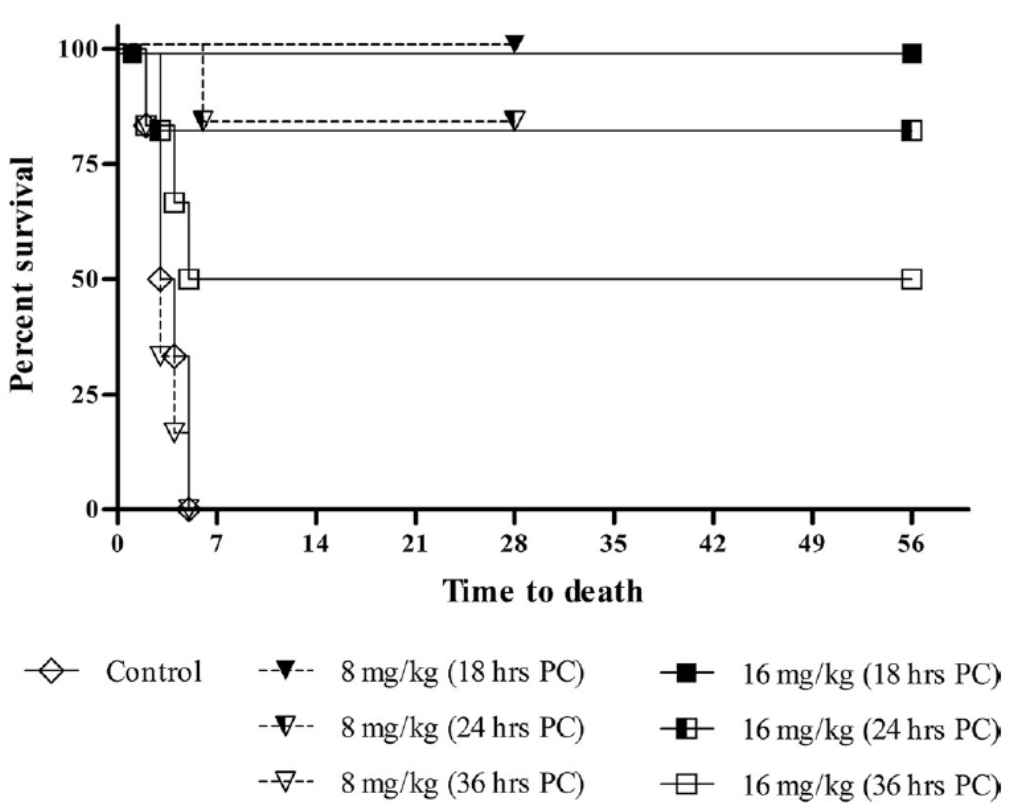
Demonstrating Efficacy of Anthrax Therapeutics in Animal Models: Pre-Exposure Prophylaxis

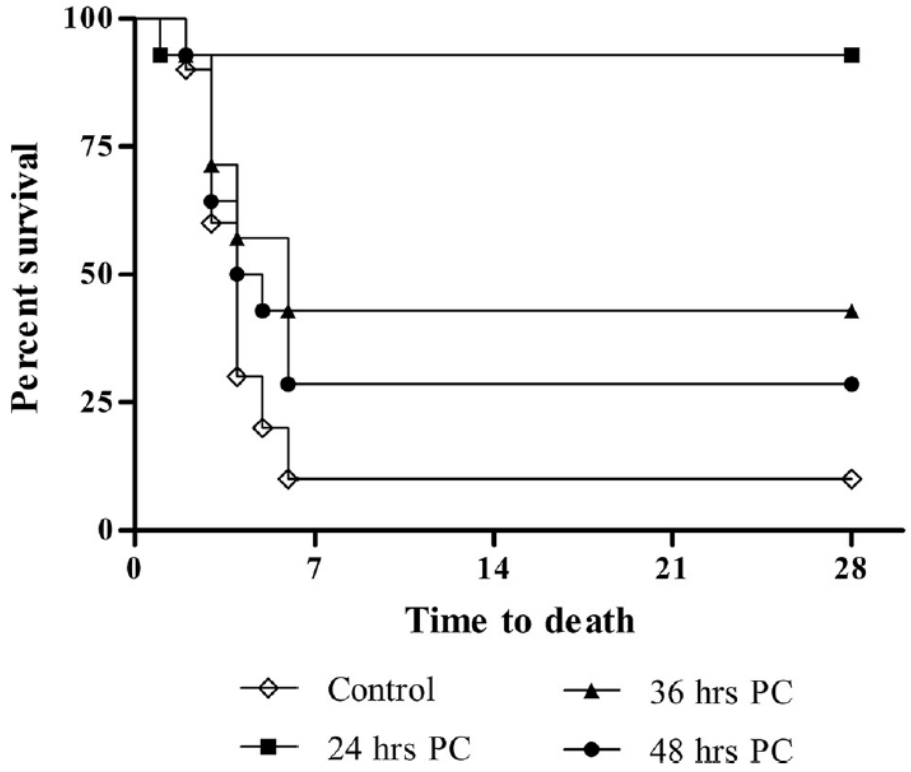


Migone et al. Raxibacumab for the treatment of inhalational anthrax. *NEJM* 2009; 361(2):135-44.

SC = subcutaneous; IV = intravenous

Demonstrating Efficacy of Anthrax Therapeutics in Animal Models: Post-Exposure Prophylaxis





PC=post-challenge

Yamamoto et al. Obiltoxaximab prevents disseminated *Bacillus anthracis* infection and improves survival during pre- and postexposure prophylaxis in animal models of inhalational anthrax. *Antimicrob Agents Chemother.* 2016; 60(10):5796-805.

Demonstrating Efficacy of Anthrax Therapeutics in Animal Models: Therapeutic Intervention

Rabbit efficacy study survival results

	All animals		Animals bacteremic prior to treatment	
AVP-21D9 dose (mg/kg)	No. survived/ no. dosed	Survival proportion (95% confidence interval)	No. survived/ no. dosed	Survival proportion (95% confidence interval)
20	9/12	0.75 (0.43, 0.95)	6/9	0.67 (0.30, 0.93)
10	13/14	0.93 (0.66, 1.00)	10/11	0.91 (0.59, 1.00)
5	13/14	0.93 (0.66, 1.00)	12/13	0.92 (0.64, 1.00)
1	8/14	0.57 (0.29, 0.82)	3/9	0.33 (0.07, 0.70)
0 (saline)	0/8	0.00 (0.00, 0.37)	0/6	0.00 (0.00, 0.46)

NHP efficacy study survival results

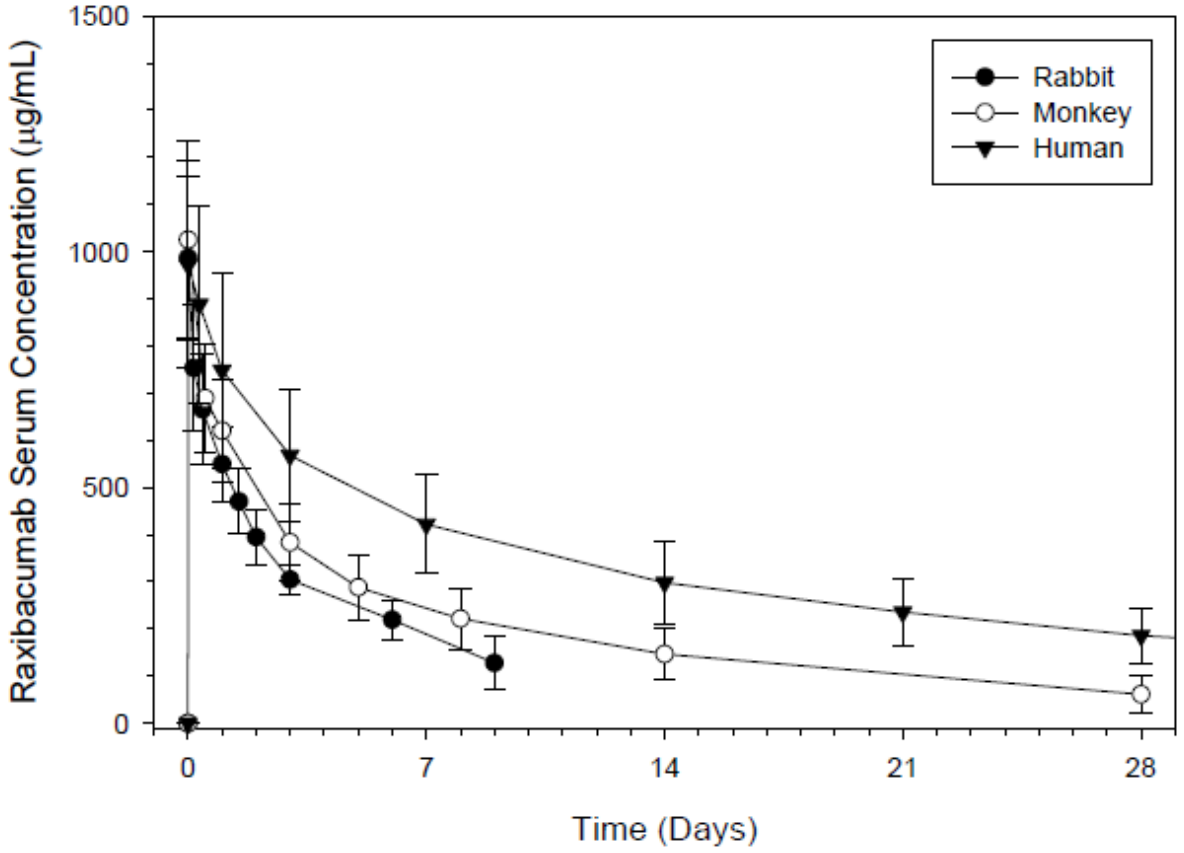
	All animals		Animals bacteremic prior to treatment	
AVP-21D9 dose (mg/kg)	No. survived/ No. dosed	Survival proportion (95% confidence interval)	No. survived/ no. dosed	Survival proportion (95% confidence interval)
20	6/10	0.60 (0.26, 0.88)	6/10	0.60 (0.26, 0.88)
10	4/10	0.40 (0.12, 0.74)	4/10	0.40 (0.12, 0.74)
5	7/10	0.70 (0.35, 0.93)	6/9	0.67 (0.30, 0.93)
1	6/10	0.60 (0.26, 0.88)	6/10	0.60 (0.26, 0.88)
0 (saline)	0/8	0.00 (0.00, 0.37)	0/8	0.0 (0.00, 0.37)

- **Animals treated on individual basis upon detection of clinical sign of disease:**
  - Rabbits treated based on fever*
  - NHPs treated based on serum PA*
- **Bacteremia confirmed retrospectively by culture:**
  - Recommended by FDAas biomarker for therapeutic intervention**
  - FDA's primary analysis uses only animals bacteremic at the time of treatment**

* Malkevich et al. Efficacy and safety of AVP-21D9, an anthrax monoclonal antibody, in animal models and humans. *Antimicrob Agents Chemother.* 2014; 58(7):3618-25.

** Center for Drug Evaluation and Research, FDA. Briefing package: raxibacumab. *Anti-infective Drugs Advisory Committee Meeting.* 2009. https://wayback.archive-it.org/7993/20170113045235/http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/Anti-InfectiveDrugsAdvisoryCommittee/ucm187299.htm

Bridging Animal and Human Pharmacokinetics



Mean (SD) serum concentration-time profiles for raxibacumab following single intravenous administration of 40 mg/kg in:

- Healthy male and female subjects
- Monkeys with inhalation anthrax, and
- Rabbits with inhalation anthrax

Center for Drug Evaluation and Research, FDA. Briefing package: raxibacumab. *Anti-infective Drugs Advisory Committee Meeting.* 2009. https://wayback.archive-it.org/7993/20170113045235/http://www.fda.gov/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/Anti-InfectiveDrugsAdvisoryCommittee/ucm187299.htm

Demonstrating Efficacy of Anthrax Therapeutics in Animal Models: Drug-Drug Interaction

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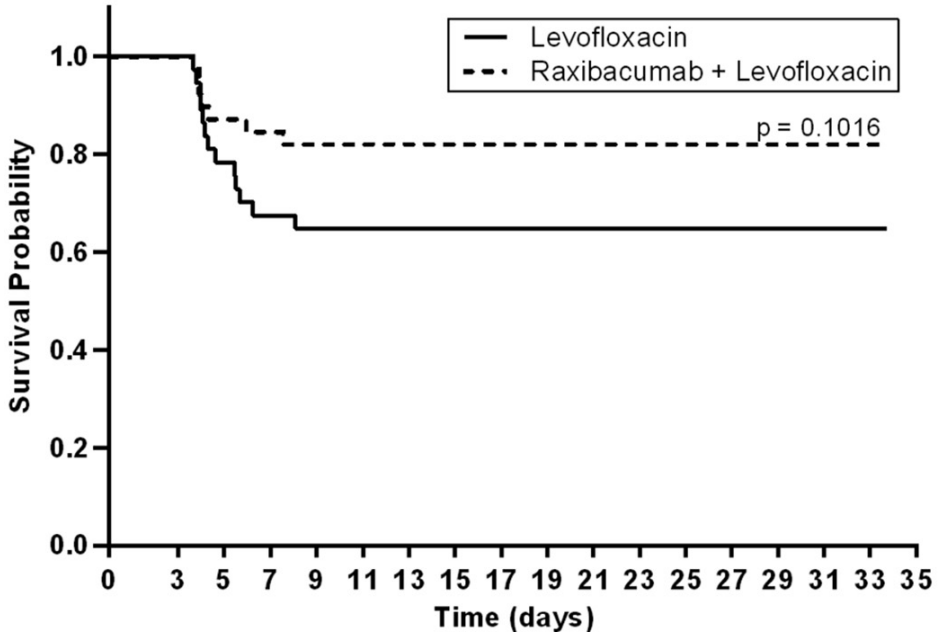

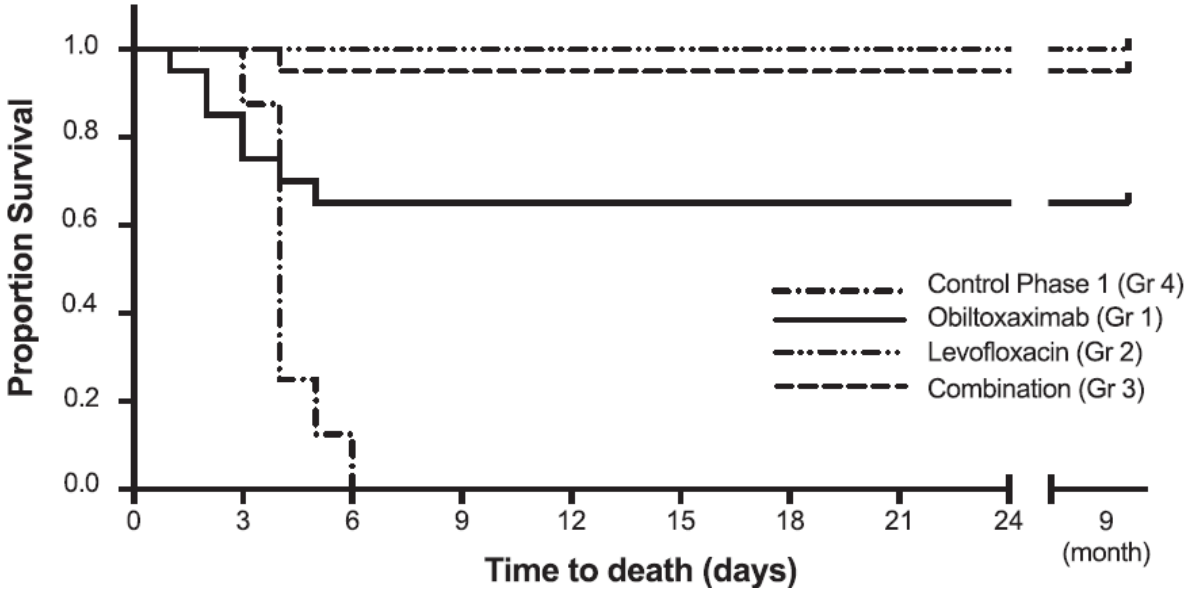

- No interference of mAb with antimicrobial treatment
- Antimicrobials highly efficacious; difficult to demonstrate added benefit
- Added benefit may be demonstrated when treatment is delayed; not statistically significant

- Migone et al. Added benefit of raxibacumab to antibiotic treatment of inhalational anthrax. *Antimicrob Agents Chemother*. 2015; 59(2):1145-51.
- Henning et al. Development of protective immunity in New Zealand White Rabbits challenged with *Bacillus anthracis* spores and treated with antibiotics and obiltoxaximab, a monoclonal antibody against protective antigen. *Antimicrob Agents Chemother*. 2018; 62(2): e01590-17.

Summary and Conclusions

- **Regulatory Pathway:**
  - Demonstrate efficacy in animals → assess drug exposure in animals
  - Evaluate pharmacokinetics in humans → assess drug exposure in humans
  - Determine drug exposure in humans required for efficacy → identify efficacious dose in humans
- **Animal Models:**
  - Rabbits (usually NZW) and NHPs (usually cynomolgus macaques) → approximate human disease
  - Pre- and post-exposure prophylaxis → proof-of-concept efficacy
  - Therapeutic intervention → demonstration of efficacy as monotherapy
    - Treatment initiated based on fever (rabbits) or toxemia (NHPs)
    - Bacteremia confirmed retrospectively
  - Drug-drug interaction:
    - Demonstration of non-interference with standard of care (required for any adjunct)
    - Assessment of added benefit compared to antimicrobials alone (desired)