Animal Models for Botulinum Antitoxins

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Agenda

INTRODUCTION
Provide a brief overview of botulinum neurotoxins (BoNTs).

ANIMAL MODELS OF BOTULISM
Examine the diversity of animal test systems that have been used for BoNTs studies.

PAST APPROACHES TO EFFICACY DEMONSTRATION
Review the previous approval pathway for a current BoNT antitoxin.

MODEL SELECTION FOR MCM PRODUCT EVALUATION
Offer considerations for human translatability from nonclinical studies.

BRIEF OPEN DISCUSSION
Answer any questions as they arise.

Main Objective

The primary purpose of today's 20-minute presentation is to provide a scientific overview of botulinum neurotoxins and discuss the role of nonclinical models used to evaluate treatments for human relevant translation.

Introduction: Botulinum Neurotoxins (BoNTs)

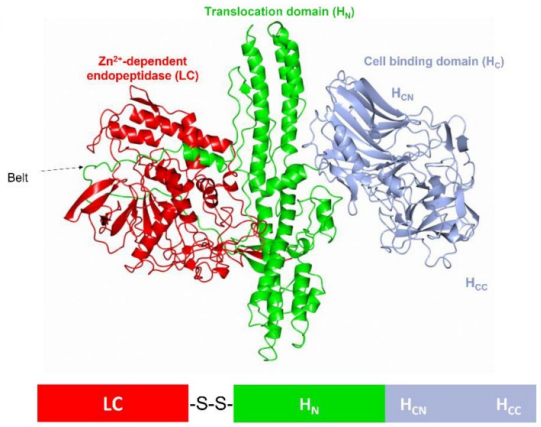
References

[1] Schiavo et al., 1994. *Semin Cell Bio*. **5**(4):221-9; [2] Pirazzini et al., 2017. *Pharmacol Rev*. **69**(2):200-235; [3] Montal, 2010. *Annu Rev Biochem*. **79**:591-617; [4] Kumar and Singh, 2025. *Int J Mol Sci*. **26**(2):777

Summary

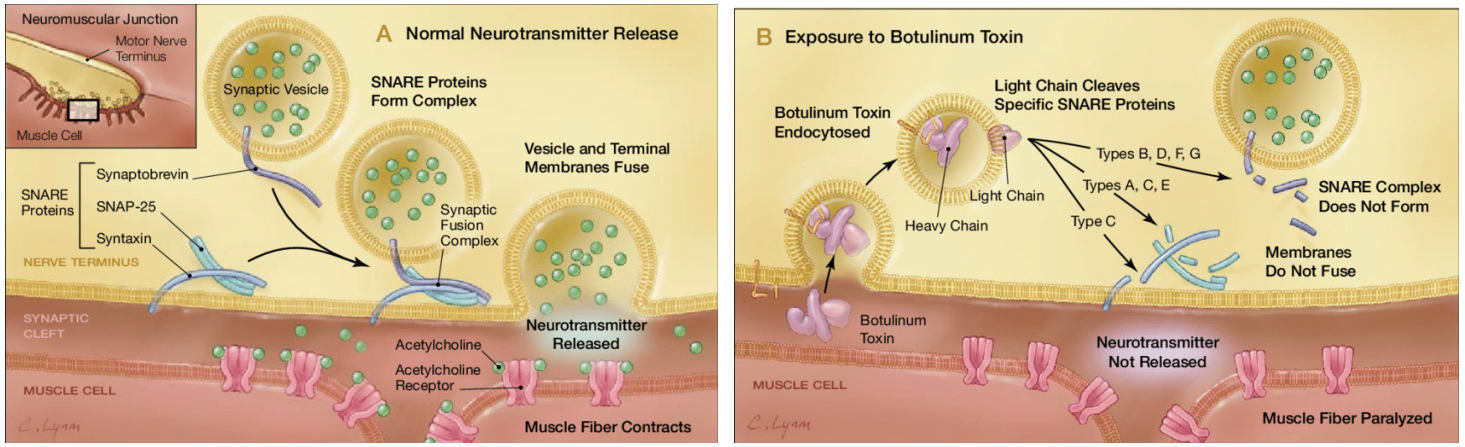
- Botulinum neurotoxins (BoNTs) are potent neuroparalytic proteins that present a significant threat to public health.
- BoNTs are the most toxic substances known, with estimated human LD_{50} values as low as 0.1-1 ng/kg.[1]
- Over 40 BoNT variants have been identified, classified into seven serotypes (BoNT/A-G) based on genetic and antigenic differences.[2]
- Structurally, each BoNT serotype consists of a 100-kDA heavy chain and a 50-kDa light chain associated through a disulfide bond.[3]
  - *Heavy chain* = neuron-specific binding, uptake via synaptic endocytosis, translocation into the presynaptic cytosol
  - *Light chain* = Zn^{2+}-dependent metalloprotease
- Proteolytic cleavage of one of three neuronal SNARE proteins blocks vesicle fusion, thereby preventing neurotransmitter release onto the postsynaptic membrane.[4]
  - SNAP-25 = BoNT/A, /C, and /E; Synaptobrevin-1/2 = BoNT/B, /D, /F, /G; Syntaxin-1 = BoNT/C

Structure of Botulinum Neurotoxin



Gregory and Acharya, 2023. *Toxins*. **15**(2):92

Molecular Action of Botulinum Neurotoxin



Arnon et al., 2001. *JAMA*. **258**(8):1059-1070

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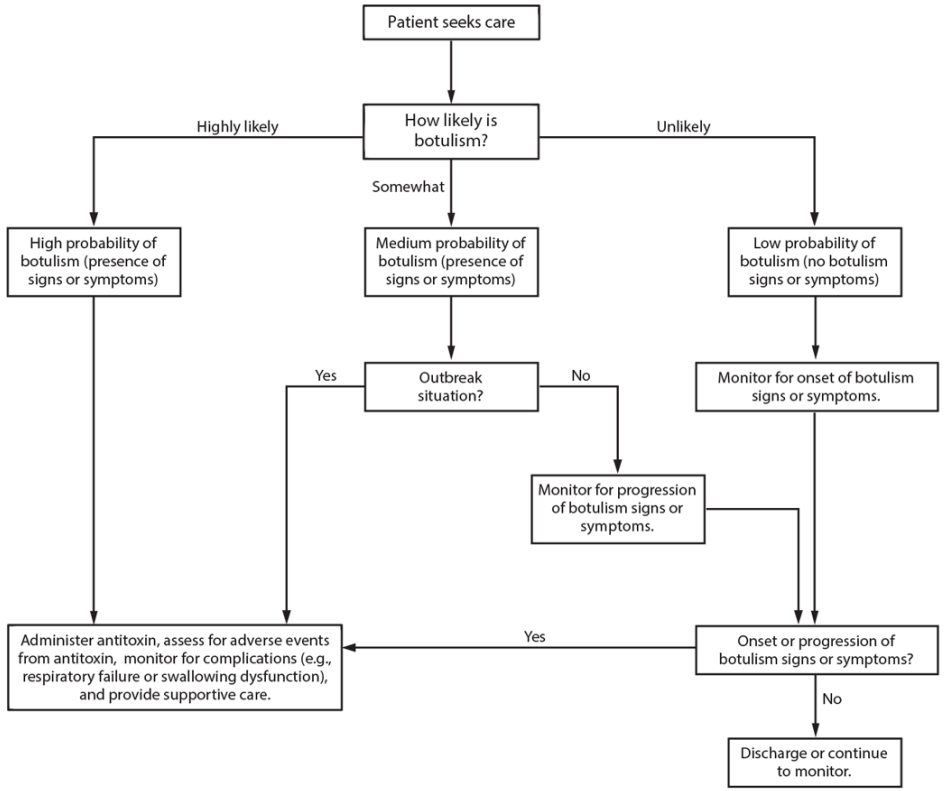
Introduction: Botulism

Summary

- Botulism is the clinical disease caused by BoNTs. Exposure to BoNTs can occur through foodborne ingestion, wound colonization, gut colonization, inhalation, and injection (iatrogenic botulism).[1]
- Blockade of neuromuscular function typically manifest as a descending flaccid paralysis.[2] Severe exposures can transition to respiratory failure within 3 days as muscles involved in sustaining respiration become functionally paralyzed.[3]
- If respiratory function is compromised, survival requires artificial ventilation and parenteral nutrition until respiratory paralysis is resolved.
- Death typically occurs from early respiratory failure or complications from prolonged intensive care.[3]
- The only approved treatment for botulism in adults is with equine-derived immunoglobulin antitoxins[4]. BabyBIG® (Botulism Immune Globulin (Human)) is an orphan drug approved for infant botulism[5].
- Antitoxins work by binding and neutralizing BoNT in the bloodstream but cannot affect toxin already bound to neuronal receptors or internalized into neurons.
- Many patients given antitoxin after symptomatic emergence still require artificial ventilation and enteral nutrition for survival.[4]

Clinical Guidelines for Botulism: Patient assessment with known or possible exposure to BoNT[1]



References

[1] Rao et al., 2021,. *MMWR Recomm Rep*. **70**(No.RR-2):1-30; [2] Lindstrom and Korkeala, 2006. *Clin Microbiol Rev*. **19**(2):298-314; [3] Witoonpanich 2010. *Clin Toxicol (Phila)*. **48**(3):177-183; [4] Richardson et al., 2020. *Clin Infect Dis*. **70**(9):1950-1957; [5] Morris et al., 2022. *J Edu Teach Emerg Med*. 7(2):S48-77

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Animal Models of Botulism: Toxicity

Summary

- The variety of laboratory animal test systems utilized for the study of BoNTs over the past 100 years is extensive.
- The most routinely utilized species include[1]:
  - Mice
  - Rats
  - Guinea Pigs
  - Rabbits
  - Nonhuman Primates
- Parenteral injection (IP, IV, IM, SC) generally represents the most commonly utilized exposure route in animal studies.
  - The primary advantages of parenteral dosing include cost, ease, toxin material requirements, safety, throughput, and reduced variability[2].
- However, animal model characterization for oral and inhalation exposures have also been performed[1-2].
- The toxic sensitivity of animals to BoNT exposure can widely vary depending on:
  - Species; BoNT serotype; Exposure route

Comparative toxicity of BoNTs in animal models[1]

BoNT Type	Source	Mouse	Rat	Guinea Pig	Rabbit	Dog	Cat	Monkey	Fowl	Pigeon	Turkey	Zebra Fish *
A	[50]	1	2.5									
	[51]	1		0.5	0.3				15			
	[52]	1			0.8 (i.m.)							
	[53]	1						0.78 (i.m.)				
	[54]	1						0.5 (i.v.)				
	[55]	1						11 (inh.)				
	[56]	1										100 (ic.)
B	[50]	1	1000									
	[52]	1			0.1 (i.m.)							
	[55]	1						432 (inh.)				
	[57]	1						150 (inh.)				
	[58]	1		0.2								
	[59]	1		0.3								
C	[60]	1	6	1	0.1	1.000	800	0.3	2000	20		
	[61]	1									7 (i.v.)	
	[56]	1										400 (ic.)
D	[60]	1	320	0.2	0.2	100.000	15.000	100	100.000	2000		
	[56]	1										20 (ic.)
E	[60]	1	40	0.5	1	100	400	1	25	25		
F	[62]	1						0.5 (s.c.)				

* IC50 immobilizing dose intracelomatic injection; i.m., intramuscular; i.v., intravenous; s.c., subcutaneous; inh., inhalation; ic., intracelomatic.

References

[1] Rossetto and Montecucco, 2019. Toxins. **11**(12):686; [2] Sanford et al., 2010. Clin Vaccine Immunol. 17(19):1293-1304.

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Animal Models of Botulism: Assays vs. Efficacy Evaluation

Mouse Potency or Mouse Lethality Assay (MPA or MLA)[1]

- Purpose: to measure the potency (activity) in mouse intraperitoneal LD_{50} units per mL ($MIPLD_{50}$/mL) of BoNT in various sample matrices including:
  - Pharmaceutical formulations.
  - Environmental or food samples.
  - Characterization of research-grade toxin.
- Method: a test dilution series is performed across a range of BoNT concentrations. Dilutions are dosed by the IP route at a volume of 0.5 mL. Mortality observations are performed at ~96 hours after dosing.
- Result: LD_{50} (expressed in $MIPLD_{50}$/mL) calculated from a mortality distribution using probit analysis.

Mouse Neutralization Assay (MNA)[2]

- Purpose: to measure the amount of BoNT neutralized per unit of neutralizing antibody in a sample to determine antibody strength in samples including:
  - Confirm serospecificity of BoNT samples using specific antitoxin reference standards.
  - Determine concentrations of products or samples containing antibodies to BoNT.
  - Sample analysis to support clinical and non-clinical studies.
- Method: varies, but dilutions of standard/test curves can be pre-incubated with toxin which is dosed and monitored similar to the MPA.
- Result: ED_{50} calculated from a mortality distribution using probit analysis.

vs.

Nonclinical Efficacy Evaluation

- Purpose: to translate predicted relevant benefit of a candidate MCM to humans from data that is derived from well-controlled nonclinical studies performed in well-characterized animal models of a target disease.
- Method: product specific
- Result: product specific

Example case study to be discussed in the subsequent slides

References

[1] Pearce et al., 1994. **128**(1):69-77; [2] Center for Disease Control, 1987. *Clostridium botulinum monovalent and polyvalent antitoxins.*

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Past Approaches to Nonclinical Efficacy Evaluation

Current (US) Antitoxin Background

- In 2006, the US Department of Health and Human Services awarded a $363 million contract to Cangene Corporation (now Emergent) for delivery of 200,000 doses of Botulism Antitoxin Heptavalent (BAT®) over five years for delivery into the US Strategic National Stockpile (SNS)[1].
- BAT® (formerly known as HBAT) was developed from equine plasma with funding from BARDA and was approved in 2013 by the US Food and Drug Administration (FDA) as the first MCM product to treat all known BoNT serotypes* associated with botulism.
  - *Note: labeled for BoNT/A through /G
- The decision to approve BAT® was based entirely on nonclinical efficacy studies conducted in animal models of botulism[2] using the US FDA Animal Rule[4]

What nonclinical animal models of botulism were used for approval?

Prescribing Information for BAT® [2]

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use BAT® safely and effectively. See full prescribing information for BAT®.

BAT® [Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) - (Equine)] Sterile Solution for Injection
Initial U.S. Approval: 2013

----------RECENT MAJOR CHANGES----------
Dosage and Administration, Preparation (2.2) [09/2016]

----------INDICATIONS AND USAGE----------
BAT [Botulism Antitoxin Heptavalent (A, B, C, D, E, F, G) – (Equine)] is a mixture of immune globulin fragments indicated for the treatment of symptomatic botulism following documented or suspected exposure to botulinum neurotoxin serotypes A, B, C, D, E, F, or G in adults and pediatric patients.
The effectiveness of BAT is based solely on efficacy studies conducted in animal models of botulism.

----------DOSAGE AND ADMINISTRATION----------
For intravenous use only.

Administer BAT by slow intravenous infusion after dilution 1:10 in normal saline at the dose recommended in the following table.

Patient Group	Dose	Starting Infusion Rate (first 30 minutes)	Incremental Infusion Rate if Tolerated (every 30 minutes)	Maximum Infusion Rate
Adults (≥ 17 years)	One vial	0.5 mL/min	Double the rate	2 mL/min
Pediatric (1 year to <17 years)	20 – 100% of adult dose	0.01 mL/kg/min Do not exceed the adult rate	0.01 mL/kg/min	0.03 mL/kg/min Do not exceed the adult rate
Infants (< 1 year)	10% of adult dose regardless of body weight	0.01 mL/kg/min	0.01 mL/kg/min	0.03 mL/kg/min

----------DOSAGE FORMS AND STRENGTHS----------
Each single-use vial contains a minimum potency of:

- 4,500 Units (U) for serotype A antitoxin,
- 3,300 U for serotype B antitoxin,
- 3,000 U for serotype C antitoxin,
- 600 U for serotype D antitoxin,
- 5,100 U for serotype E antitoxin,
- 3,000 U for serotype F antitoxin, and
- 600 U for serotype G antitoxin

----------CONTRAINDICATIONS----------
None.

----------WARNINGS AND PRECAUTIONS----------

- Hypersensitivity reactions including anaphylaxis. Prepare for monitoring and management of allergic reactions (5.1).
- Delayed allergic reactions (serum sickness). Patient monitoring is recommended (5.2).
- Infusion reactions. Monitor and slow or interrupt infusion and administer treatment based on the severity of the reaction (5.3).
- Interference with non-glucose specific blood sugar testing systems. Use glucose-specific testing systems (5.4).
- BAT is made from equine plasma and may contain infectious agents e.g. viruses (5.5).

----------ADVERSE REACTIONS----------

- The most common adverse reactions observed in ≥5 % of healthy volunteers in clinical trials were headache, nausea, pruritus, and urticaria (6.1).
- The most common adverse reactions reported in ≥1% of patients in a clinical study were pyrexia, rash, chills, nausea, and edema (6.1).
- One serious adverse reaction of hemodynamic instability was observed in one patient in the clinical study (6.1).

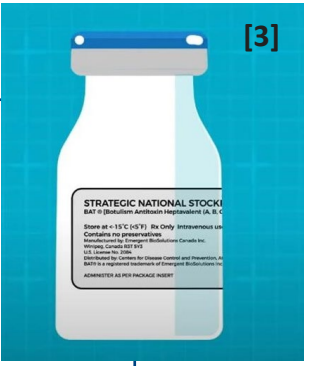
To report SUSPECTED ADVERSE REACTIONS, contact Cangene Corporation at 1-800-768-2304 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

----------USE IN SPECIFIC POPULATIONS----------

- Pediatric: Limited safety data is available in the pediatric population. Dosing in pediatric patients is based on Salisbury Rule (8.4).

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling

Revised: 03/2017



References

[1] Press Release: https://www.biospace.com/b-cangene-corporation-b-is-awarded-us-362-million-supply-contract-by-u-s-government-for-anti-botulism-drug; [2] https://www.fda.gov/media/85514/download; [3] https://www.cdc.gov/botulism/treatment/index.html; [4] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/product-development-under-animal-rule

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Past Approaches to Nonclinical Efficacy Evaluation (GPs)

Guinea Pig Model [1]

- A challenge dose optimization study performed in Hartley guinea pigs
  - Male and Female; 400-500 g
  - Single intramuscular (IM) challenge
- The objective was to identify an IM intoxication dose for BoNT/A and BoNT/E that produced highly lethal level outcomes with an adequate window for post-exposure antitoxin rescue after onset of toxicity.
  - 4.0x GPIMLD$_{50}$ disease progression was too rapid and severe for BAT® rescue after onset of disease
- 10 animals/group; gender balanced:
  - BoNT/A = 1.5x, 2.0x, or 4.0x GPIMLD$_{50}$
  - BoNT/E = 1.5x, 2.0x, or 4.0x GPIMLD$_{50}$
  - 1 vehicle control group (saline)
- Animal observations were performed hourly (BoNT/A) or half-hourly (BoNT/E) during dynamic period of disease.

Table 3. Kaplan-Meier median (95% confidence interval) for time to onset of clinical signs for animals challenged with BoNT serotypes A and E and control group.

Clinical Sign	Kaplan-Meier Median (95% Confidence Interval) Time to Onset of Clinical Signs (Hours) in Each Study Group						
	Serotype A			Serotype E			Control
	1.5x GPIMLD$_{50}$	2x GPIMLD$_{50}$	4x GPIMLD$_{50}$	1.5x GPIMLD$_{50}$	2x GPIMLD$_{50}$	4x GPIMLD$_{50}$	PBS
Lethargy[1]	74 (71, 81)	45 (45, 59)	31 (27, 42)	24 (21, 26)	22 (21, 28)	(17, —)	—
Salivation[2]	73 (67, 75)	55 (52, 71)	39 (36, 49)	50 (29, 50)	33 (33, —)	24 (19, 24)	—
Lacrimation[2]	109 (103, 127)	74 (67, 89)	51 (46, —)	72 (—)	51 (28, 51)	26 (21, 26)	—
Weakness of the Right Hind Limb Only[2]	41 (34, 46)	27 (27, 31)	24 (21, 25)	15 (13, 17)	16 (13, 18)	13 (12, 13)	—
Weak Limbs[2]	61 (52, 69)	45 (40, 48)	32 (27, 37)	21 (20, 24)	20 (17, 24)	15 (14, 17)	—
Noticeable Change in Breathing Sound Rate or Pattern[2]	48 (36, 48)	35 (33, 36)	29 (29, 32)	22 (21, 24)	19 (15, 21)	14 (13, 16)	—
Forced Abdominal Respirations[3]	153 (125, 165)	74 (69, 87)	43 (41, 45)	30 (26, 39)	26 (24, 30)	19 (17, 20)	—
Total Paralysis[3]	165 (165, —)	78 (69, 97)	45 (45, 47)	32 (30, 41)	29 (26, 34)	21 (20, 22)	—
Any Moderate Sign	37 (32, 42)	27 (27, 31)	24 (21, 25)	14 (10, 17)	16 (12, 18)	13 (12, 13)	—
Any Severe Sign	153 (125, 165)	72 (69, 87)	43 (41, 45)	30 (26, 39)	26 (24, 30)	19 (17, 20)	—
First Clinical Sign	37 (32, 42)	27 (27, 31)	24 (21, 25)	14 (10, 17)	16 (12, 18)	13 (12, 13)	—

Table 4. Summary of Kaplan-Meier median (95% confidence interval) duration of clinical signs (therapeutic window) and mean and median time to death for BoNT serotypes A and E.

Serotype	Group	Median Duration in Hours of Clinical Signs (Range)	Mean Time to Death in Hours (Range)	Median Time to Death in Hours (95% Confidence Interval)
A	1.5x GPIMLD$_{50}$	121 (72, 129)	143 (102, 165)	165 (102, 165)
	2x GPIMLD$_{50}$	63 (52, 71)	92 (69, 143)	92 (79, 99)
	4x GPIMLD$_{50}$	26 (25, 34)	52 (46, 66)	51 (47, 57)
E	1.5x GPIMLD$_{50}$	21 (12, 24)	39 (25, 95)	32 (30, 39)
	2x GPIMLD$_{50}$	14 (11, 19)	31 (15, 57)	30 (28, 32)
	4x GPIMLD$_{50}$	8 (7, 9)	21 (17, 28)	20 (20, 22)
Control	—	—	—	—

Key Results [1]

- Clinical observations were consistent with key aspects of human disease.
- A toxin dose of 1.5x GPIMLD50 was selected for use in therapeutic efficacy as it produced a prolonged clinical course (amenable to rescue) prior to mortality.

References

[1] Barker D., Gillum K.T., Niemuth, N.A., Kodihalli, S. 2019. Therapeutic efficacy of equine botulism heptavalent antitoxin against all seven botulinum neurotoxins in symptomatic guinea pigs. *PLoS ONE*. **14**(9): e0222670

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Past Approaches to Nonclinical Efficacy Evaluation (GPs)

Pivotal Demonstration of GP Efficacy [1]

- Randomized, blinded, and well-controlled GLP Study with 616 GPs in 14 gender-balanced groups (n=34/group)
- Challenge performed by single IM injection (0.1 mL) into the muscles of the right hind leg.
  - All toxin doses verified by MPA
- BoNT serotypes /A, /B, /C, /D, /E, /F, and /G were evaluated.
- Trigger-to-treat with a single dose of a scaled human dose of BAT® (or placebo) was defined as a 4th consecutive occurrence of a moderate or severe clinical sign.
- Single treatment was administered IV through an indwelling venous catheter.
- Primary endpoint was an enhancement of survival (to 21 days) with 1x scaled human dose of BAT® compared to placebo controls.
- Clinical observations were also collected and analyzed for effect.

Table 5. Summary of survival with fisher's exact test comparisons and Kaplan-Meier median time to death with log-rank test comparisons between BAT product-treated (1x scaled human dose) and placebo control groups in guinea pigs intoxicated with 1.5x GPIMLD$_{50}$ BoNT serotypes A, B, C, D, E, F and G.

BoNT Serotype	Group	Treatment Dose Level	Median Time to Treatment (Min, Max)[4] in Hours	Survival (percent)	Two-Sided Fisher's Exact Test Comparison (p-value)	Kaplan-Meier Median Time to Death (95% Confidence Interval) in Hours	Log-Rank Test Time-to-Death Comparison (p-value)
A	A1	1.0x BAT Product	17 (15, 23)	34/34 (100%)	<0.0001*	—(—)	<0.0001*
	A2	Placebo Control[2]	17 (16, 29)	0/34 (0%)		99 (87, 113)	
B	B1	1.0x BAT Product[1]	26 (20, 29)	34/34 (100%)	<0.0001*	—(—)	<0.0001*
	B2	Placebo Control[2]	25 (19, 29)	1/34 (3%)		94 (94, 112)	
C	C1	1.0x BAT Product[1]	22 (12, 26)	33/34 (97%)	<0.0001*	—(—)	<0.0001*
	C2	Placebo Control[2]	22 (12, 26)	4/34 (12%)		114 (111, 141)	
D	D1	1.0x BAT Product[1]	24 (22, 37)	33/34 (97%)	<0.0001*	—(—)	<0.0001*
	D2	Placebo Control[2]	24 (22, 37)	5/34 (15%)		156 (141, 180)	
E	E1	1.0x BAT Product[1]	9 (7, 16)	34/34 (100%)	<0.0001*	—(—)	<0.0001*
	E2	Placebo Control[2]	8 (8, 10)	0/34 (0%)		29 (27, 30)	
F	F1	1.0x BAT Product[1]	15 (11, 20)	34/34 (100%)	<0.0001*	—(—)	<0.0001*
	F2	Placebo Control[2]	15 (10, 20)	4/34 (12%)		58 (45, 68)	
G	G1	1.0x BAT Product[1]	23 (15, 28)	34/34 (100%)	<0.0001*	—(—)	<0.0001*
	G2	Placebo Control[2]	22 (16, 29)	17/34 (50%)		168 (143, —)[3]	

Key Results [1]

- Statistically significant enhancement of survival was achieved at a 1x scaled human dose of BAT® (14 of 30) compared to placebo (0 of 30) for BoNT serotype A
- BAT® animals still continued to develop clinical signs but progression was slowed.

References

[1] Barker D., Gillum K.T., Niemuth, N.A., Kodihalli, S. 2019. Therapeutic efficacy of equine botulism heptavalent antitoxin against all seven botulinum neurotoxins in symptomatic guinea pigs. *PLoS ONE*. **14**(9): e0222670

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Past Approaches to Nonclinical Efficacy Evaluation (NHPs)

Nonhuman Primate Model [1]

- A study was conducted to establish the LD_{50}, define disease progression, and identify optimal triggers for therapeutic intervention studies in Rhesus macaques (*Macaca mulatta*).
  - Male and Female; 3-7 kg
  - Single intravenous (IV) challenge
- Only BoNT serotype A evaluated.
- Previous LD50 for BoNT/A was reported to be 25 $MIPLD_{50}$/kg
- 4 animals/group; gender balanced:
  - BoNT/A = 25, 40, 60, 160 $MIPLD_{50}$/kg
- Animal observations were performed hourly (BoNT/A) or half-hourly (BoNT/E) during dynamic periods of disease.
- Endpoint analysis included mortality and the median time to onset of clinical signs and time to death.

Table 2. Median time to onset of clinical signs (in hours) and 95% confidence interval for each clinical sign across toxin dose groups, and median time to death (in hours) and range.

Clinical sign	*Intravenous Toxin Dose Median clinical onset time in hours (Range)*			
	25 $MIPLD_{50}$/kg (n = 4)	40 $MIPLD_{50}$/kg (n = 4)	60 $MIPLD_{50}$/kg (n = 4)	160 $MIPLD_{50}$/kg (n = 4)
Ptosis	42 (40, 61)	30 (19, 41)	21.5 (17, 32)	17.5 (17, 19)
Muscular Weakness	51 (40, 62)	39.5 (19, 41)	30.5 (26, 36)	18.5 (16, 20)
Respiratory Distress	64.5 (62, 89)	39.5 (36, 43)	34.5 (35, 36)	19 (18, 23)
Oral Discharge	- - (102, - -)	47 (34, - -)	36 (35, 41)	- - (20, - -)
Nasal Discharge	- -	- -	- - (33, - -)	- - (20, - -)
Death*	- -	47 (40, 105)	36.5 (35, 43)	23 (20, 24)

- - Not calculable due to limited number of events (i.e. limited observations of clinical sign and/or death).

* Only median time to death and range was reported

https://doi.org/10.1371/journal.pone.0186892.t002

[No Title]

Key Results [1]

- Clinical observations were consistent with key aspects of human disease, generally characterized as a pattern of descending paralysis.
- BoNT/A dose of 1.7x LD_{50} (40 $MIPLD_{50}$/kg) was selected for use in therapeutic efficacy.

References

[1] Kodihalli S., Emanuel A., Takla T., Hua Y. Hobbs C., LeClaire R., O'Donnel D.C. 2017. Therapeutic efficacy of equine botulism antitoxin in Rhesus macaques. *PLoS ONE*. **12**(11): e0186892.

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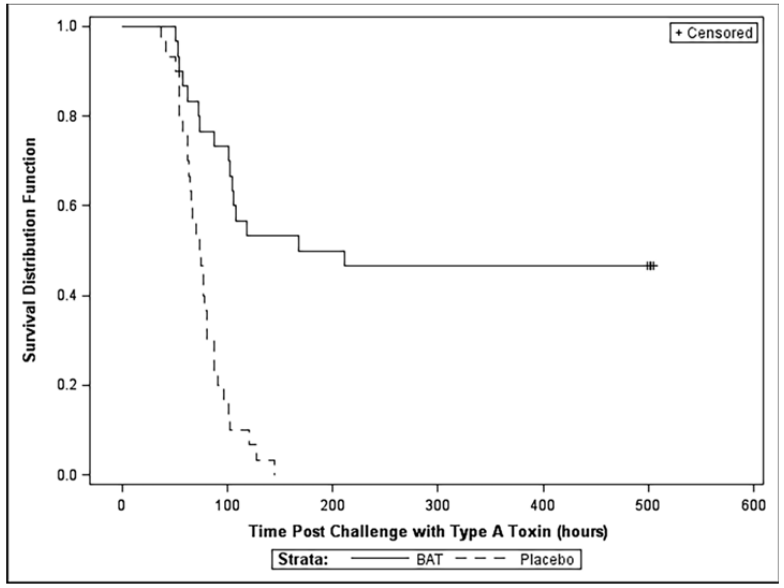
Past Approaches to Nonclinical Efficacy Evaluation (NHPs)

Pivotal Demonstration of NHP Efficacy [1]

- Randomized, blinded, and well-controlled GLP Study with 60 NHPs in 2 total gender-balanced groups (n=30/group)
- Challenge performed by single IV injection of BoNT/A (only serotype evaluated)
  - All toxin doses verified by MPA
- Trigger-to-treat with a single dose of a scaled human dose of BAT® (or placebo) was the onset of any clinical sign associated with botulism (ex: ptosis, weakness, respiratory distress, oral/nasal discharge).
- Single treatment was administered IV.
- Parenteral nutritional and hydration support provided IV after treatment.
- Primary endpoint was an enhancement of survival (to 21 days) with 1x scaled human dose of BAT® compared to placebo controls.
- Secondary efficacy included median time to death, time to onset of (severe) clinical signs, and time from onset to recovery.

Table 9. Survival and median time to death for monkeys treated with BAT at the onset of systemic disease-Therapeutic Study 2.

Treatments	Survival rate (No. of Survivors/No. in Group%)	95% Confidence Interval	Fischer's exact test (p-value)[a]	Kaplan Meier median Time to Death in Hours (95% Confidence Intervals)	Log-Rank Test (p-value)[c]
BAT	0.47 (14/30)	(0.28, 0.66)	<0.0001	189.5 (102, - -)[b]	<0.0001
Placebo Control	0.00 (0/30)	(0.00, 0.12)		74.5 (63,81)	



Key Results [1]

- Statistically significant enhancement of survival was achieved at a 1x scaled human dose of BAT® compared to placebo for all BoNT serotypes evaluated (/A through /G)
- BAT® animals still continued to develop clinical signs.

References

[1] Barker D., Gillum K.T., Niemuth, N.A., Kodihalli, S. 2019. Therapeutic efficacy of equine botulism heptavalent antitoxin against all seven botulinum neurotoxins in symptomatic guinea pigs. *PLoS ONE*. **14**(9): e0222670

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Considerations for Nonclinical Botulism Efficacy Evaluations

Yes (✓) or No (X)?	Question
✓	Does the route of BoNT exposure matter and/or is there justification for the selected challenge route?
✓	Are data available to select a BoNT challenge dose that will induce a disease severity amenable to determining efficacy?
✓	Is there a clear understanding of botulism progression in the selected animal model(s)?
✓	Does the progression of botulism in the animal model(s) align with known aspects associated with human disease?
✓	Is the animal efficacy endpoint directly related with the desired human benefit?
✓	Are the design of the animal efficacy studies situationally relevant to the intended end-use of the product?
✓	Are the design of the animal efficacy studies statistically adequate?
✓	Are the design of the animal efficacy studies well-controlled?
✓	Has a safe human dose been identified and is it scalable to animal test systems?
✓	Are there pharmacokinetics (PK) and/or pharmacodynamics (PD) data of the MCM product in the animal model(s)?
✓	Is the PK/PD in the animal model(s) altered by the disease state?
✓	Based on animal efficacy in combination with PK and/or PD in animals, is there a valid plan to select a dose and regimen in humans?

Reference: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/product-development-under-animal-rule

Main Takeaway

A well-characterized animal model with a disease condition that faithfully recapitulates the desired human endpoint(s) serves as a critical component to designing and executing translatable and situationally relevant nonclinical studies.

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It can be done

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