Preclinical evaluation of a nitric oxide-releasing prodrug as a treatment for chronic Mycobacterium abscessus infections

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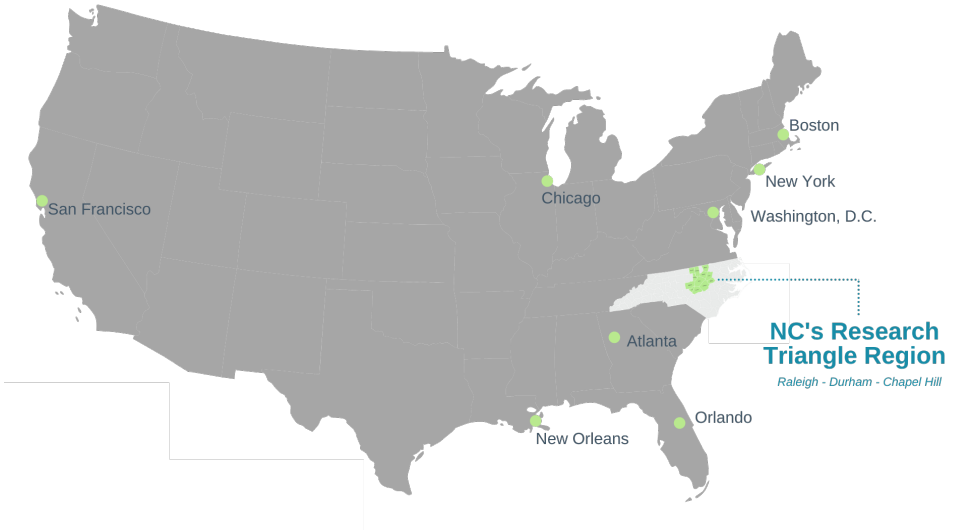
Vast Therapeutics, Inc.



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5th World Bronchiectasis & NTM Conference
June 30 - July 2, 2022

Declaration

- Mark Schoenfisch is a founder, member of the board of directors, consultant, and maintains a financial interest in Vast Therapeutics, Inc.

Company Overview

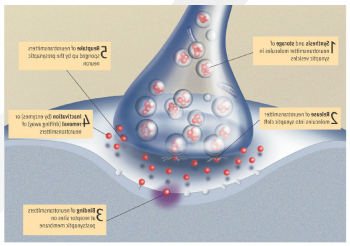
- Emerging biotech company with platform technology aimed at breaking the vicious cycle caused by **infection** and **inflammation**

Nitric oxide chemistry
+
Inhalation science

Disease specific drug candidates

Our goal is to transform the treatment of respiratory infections by:

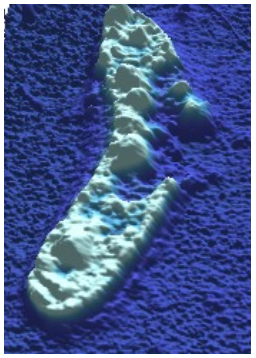
1) bringing to market a new class of antibiotic alternatives
2) redefining broad-spectrum antimicrobial activity



Immunomodulatory

Sub nM (eNOS) | Low nM (nNOS) | µM and up (iNOS)

P. aeruginosa

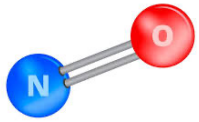


Neurotransmitter

mid nM range (nNOS)

Antibacterial

mid-nM and above (iNOS)

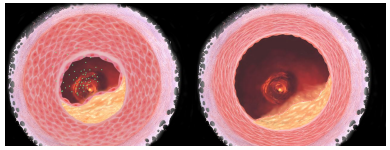


Nitric Oxide

Vasodilation

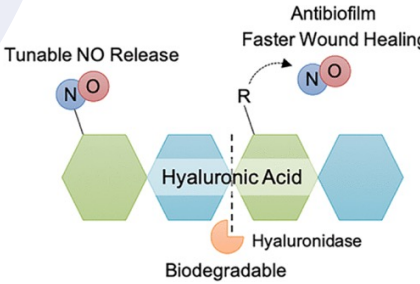
sub-pM amounts (eNOS)

Pro-Wound Healing



Promotes Angiogenesis

Inhibits Scarring



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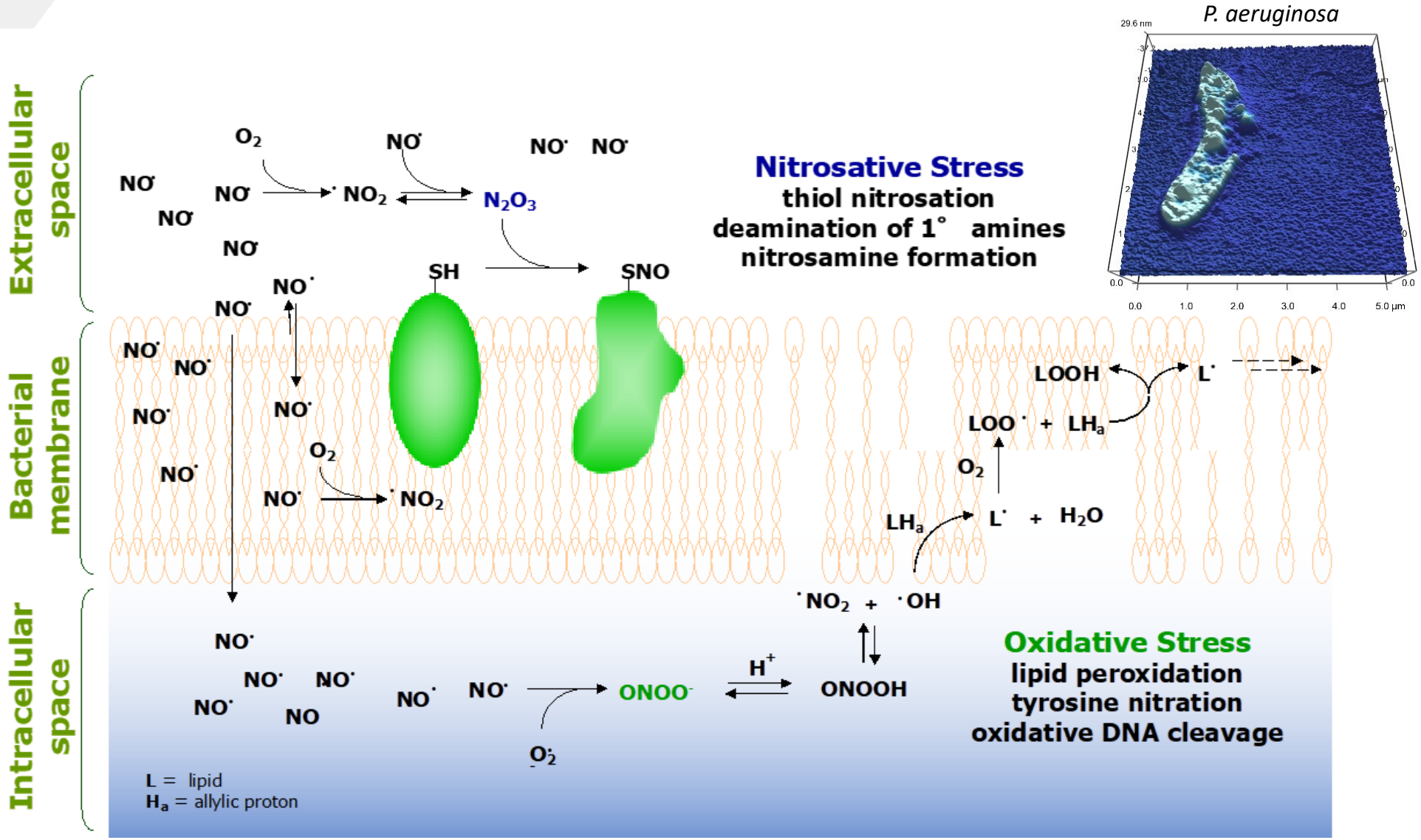
Mayer, B., Ed. *Handbook of Experimental Pharmacology; Springer, **2000**.*

Nitric Oxide Antibacterial Mechanisms of Action



Fang, F.C. (2004) *Nature Review Microbiology* 2, 830-832.
Mannick, J.B. (2006) *Proceedings of the American Thoracic Society* 3, 161-165.
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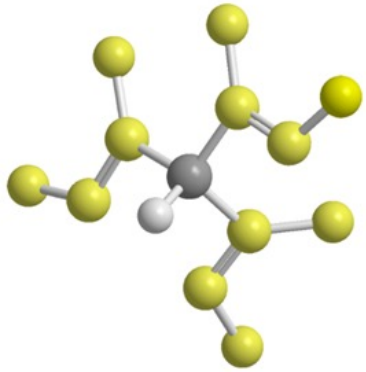
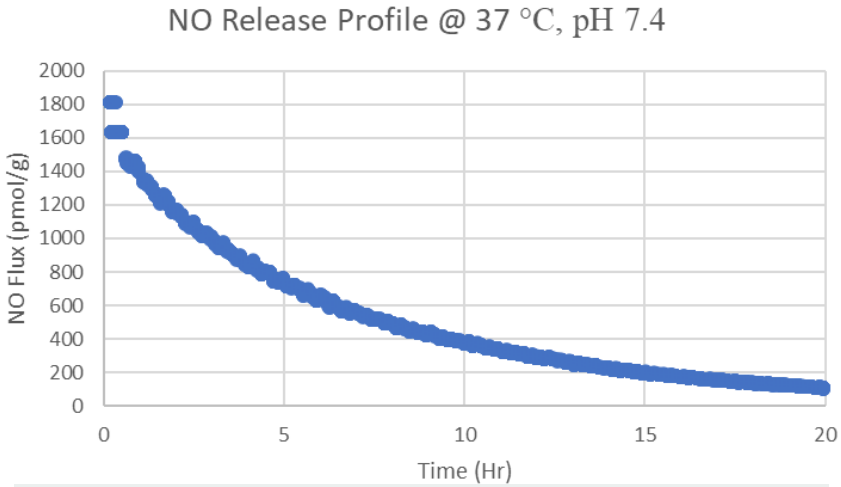
Lead Compound: MD3



- **MD3:** Low molecular weight NO-releasing compound with unparalleled antibacterial activity
- Formulated as an aqueous solution for inhalation (ALX1)
  - Release half-life of ~5 hours
  - Broad spectrum & **bactericidal**
  - Nebulized formulation (5 - 10 min treatment)
  - PARI eFlow Nebulizer



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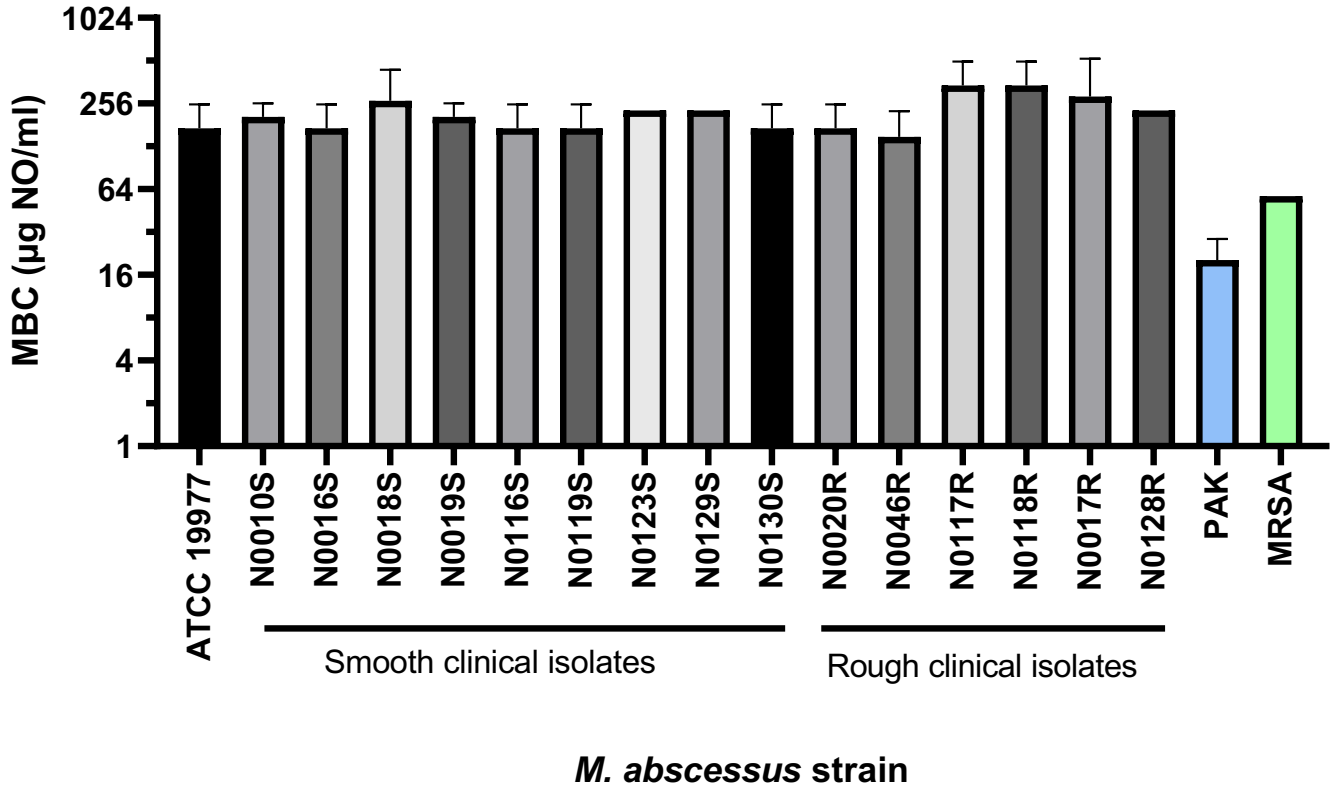
In Vitro Efficacy against *M. abscessus*

MBC assay performed using the CLSI method to evaluate efficacy of MD3 against several lab and clinical isolates of *Mycobacterium abscessus.*



Conclusions:

- MBCs are consistent regardless of *M. abscessus* isolate tested.
- MD3 activity is broad spectrum.

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MD3 Resistance Assays

¼ initial MD3, grown at 37 C shaking for 1-2 days until turbid, then repeated.

MIC determination performed in 96-well plates evaluating visual turbidity at 24-48 hours (time for negative controls to become visible).

Species	Strain	P0 (μg/mL)	P30 (μg/mL)
Mycobacterium abscessus	103 (rough)	114	114
Mycobacterium abscessus	4529 (intermediate)	57	114 (P35)
Mycobacterium abscessus	19977(smooth)	114	114 (P35)
Mycobacterium massiliense	1513 (rough)	114	114

Conclusions:

- MICs are consistent across NTM isolates tested for 30+ passages.
- Growth kinetics in broth cultures are consistent from initial culture to passage 30.
- *M. massiliense* behaved similarly indicating that decreased growth rate does not impact the effect of sub-MIC exposure on resistance.

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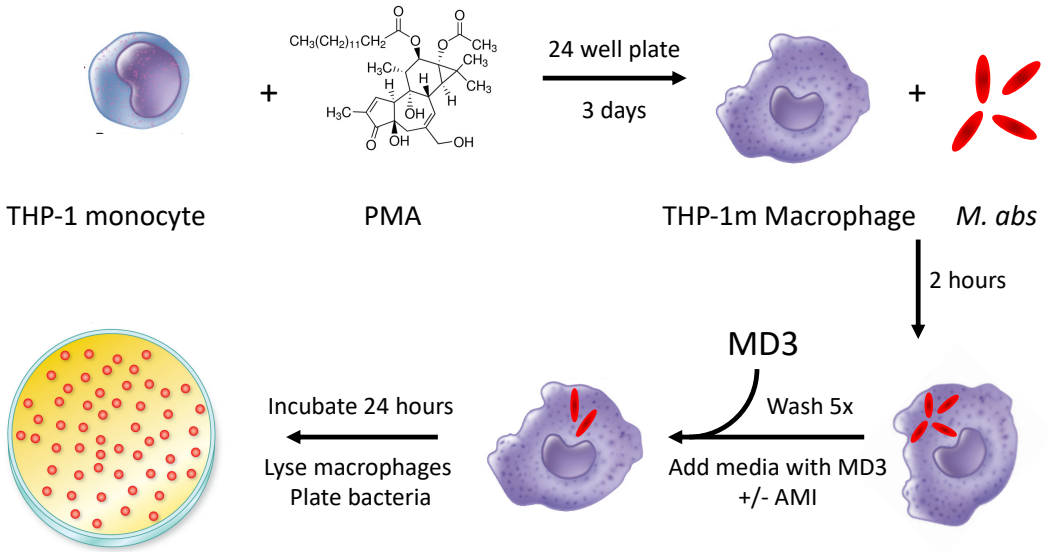
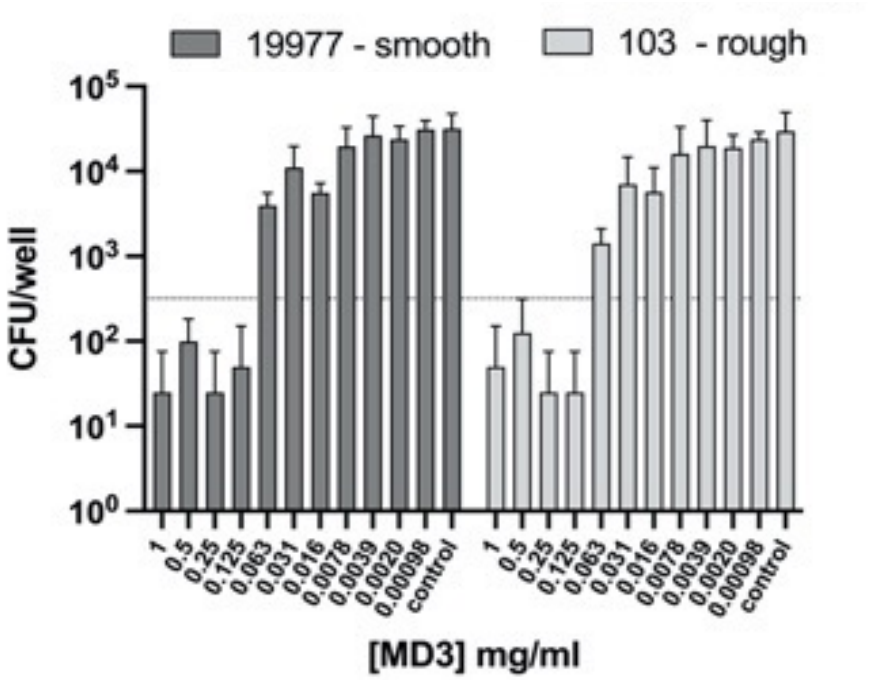
M. abscessus THP-1 Macrophage Assay





Conclusion:

- Treatment of THP-1m and phagocytosed *M. abscessus* with MD3 decreased bacterial burden in a dose dependent manner by up to 3 logs at concentrations that were non-cytotoxic to THP-1 macrophages.

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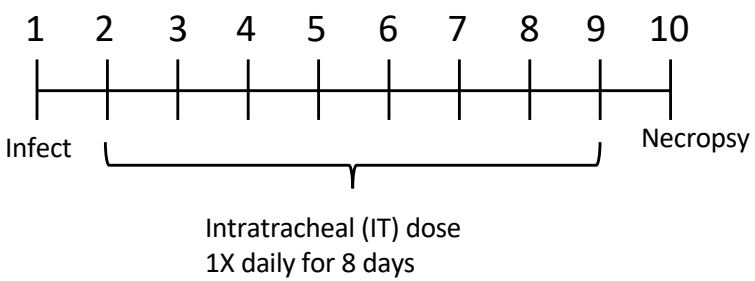
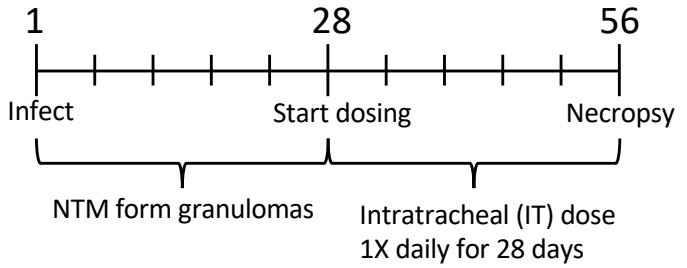
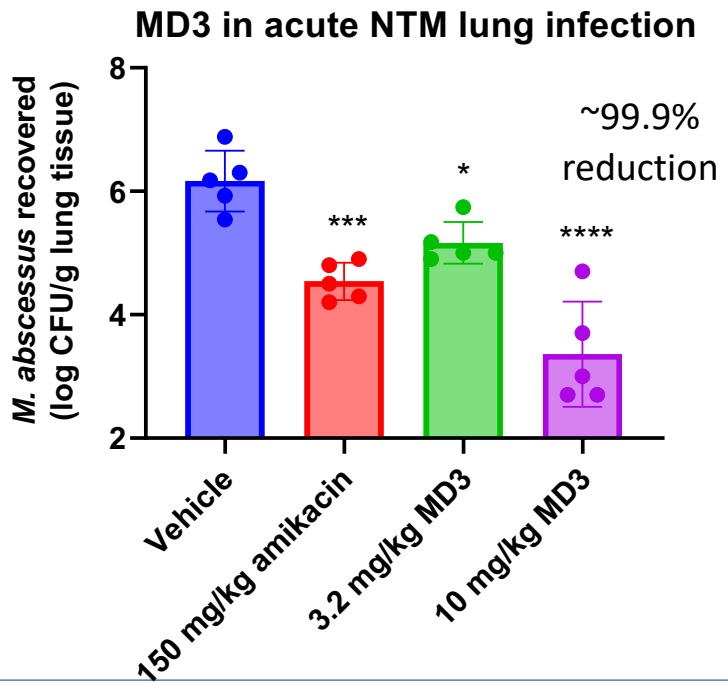
Preclinical Testing: Murine Model (Ordway; CSU)

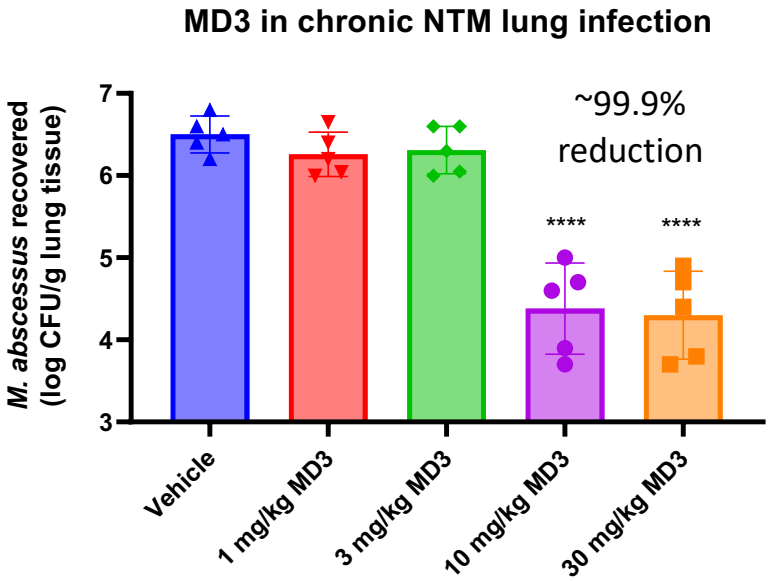
Acute NTM Infection Model



Chronic NTM Infection Model







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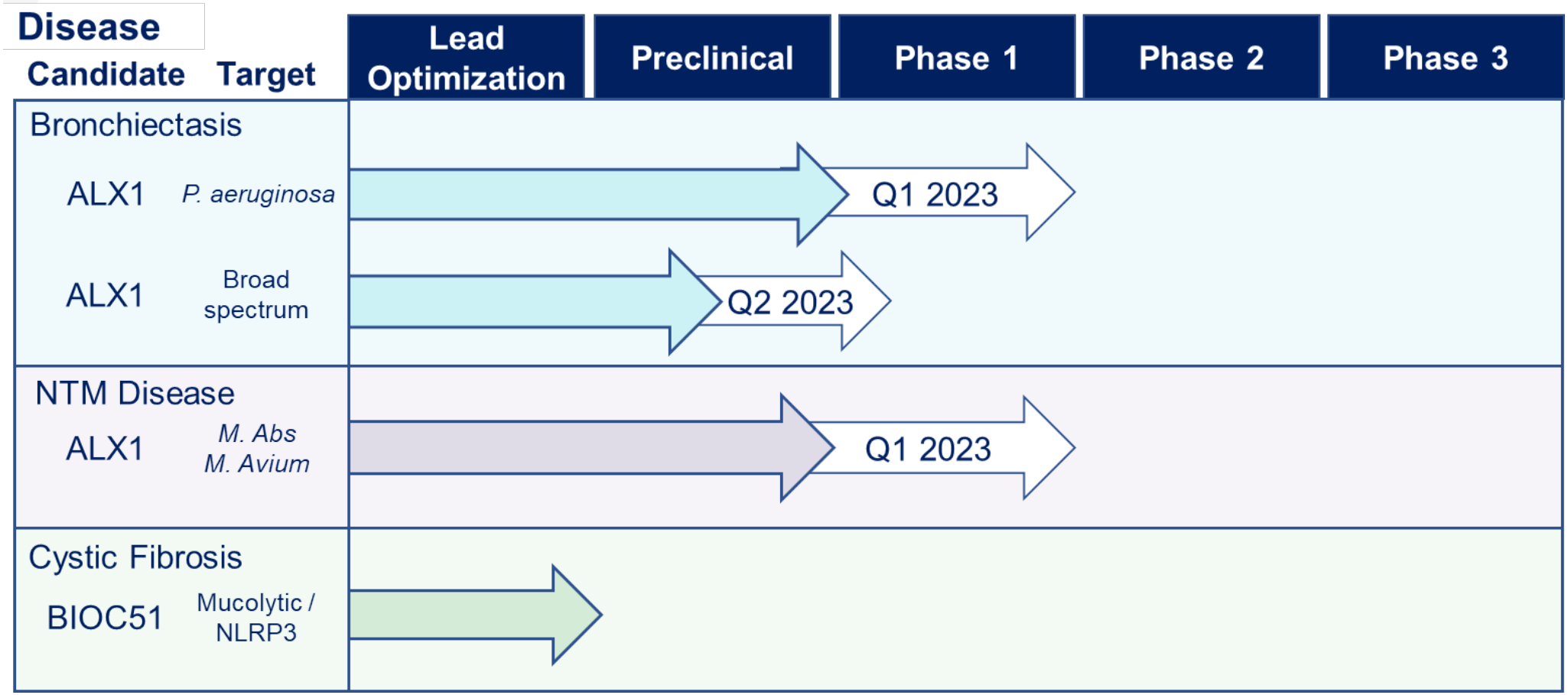
Summary & Looking Ahead

- MD3 delivers NO that results in broad spectrum antibacterial activity
- MD3 is readily nebulized as an aqueous formulation

Development milestones:

- ✓ In vivo activity against *M. abscessus*
  - ➢ Efficacy at 10 mg/kg in animal models
- ✓ In vivo activity against *P. aeruginosa*
  - ➢ Efficacy at 2 mg/kg in animal models
- ✓ IND enabling GLP toxicology program complete
- ○ Phase 1 SAD/MAD clinical program (Q3 2022)

Drug Pipeline



Leveraging a broad-spectrum platform to address chronic airway infections.

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