

# Anti-Persister Strategy for the Treatment of Chronic, Recurrent Infections

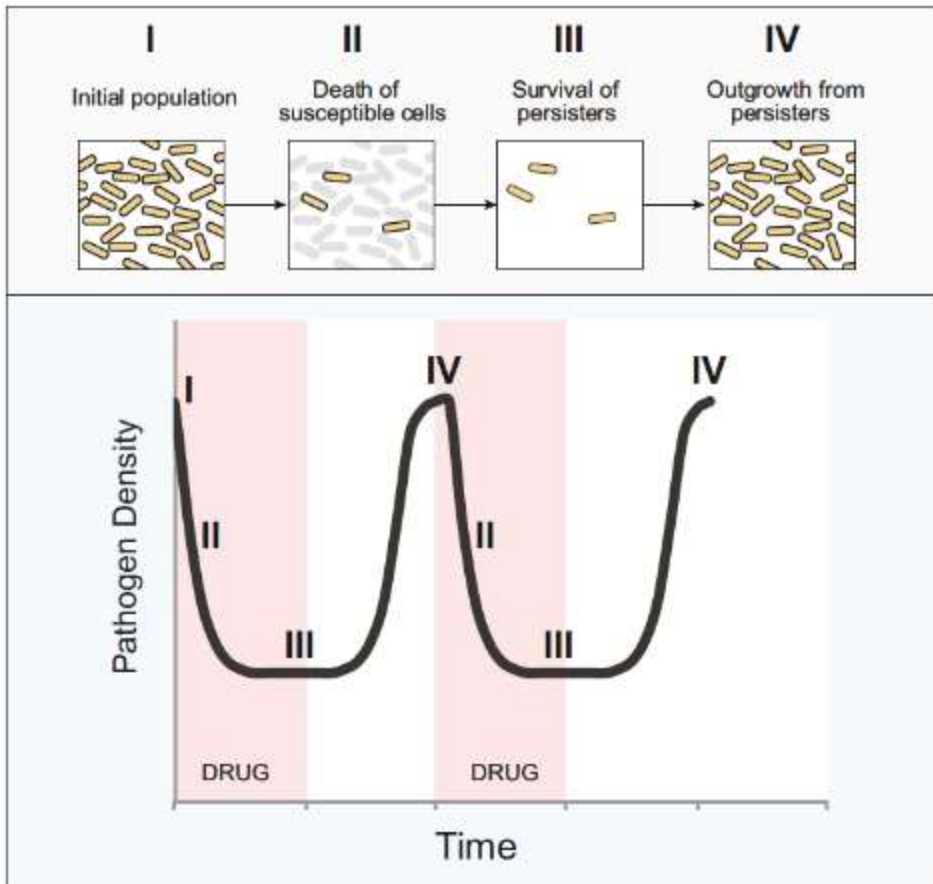
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ENBIOTIX

# The Bacterial Persister Problem



Cohen et al, *Cell Host Microbe* 2013; 13:632

## Bacterial persisters are:

- 1 Dormant, not growing
- 2 Genetically identical but tolerant to antibiotics
- 3 Responsible for recurrent infections

Tolerance

precedes

Resistance

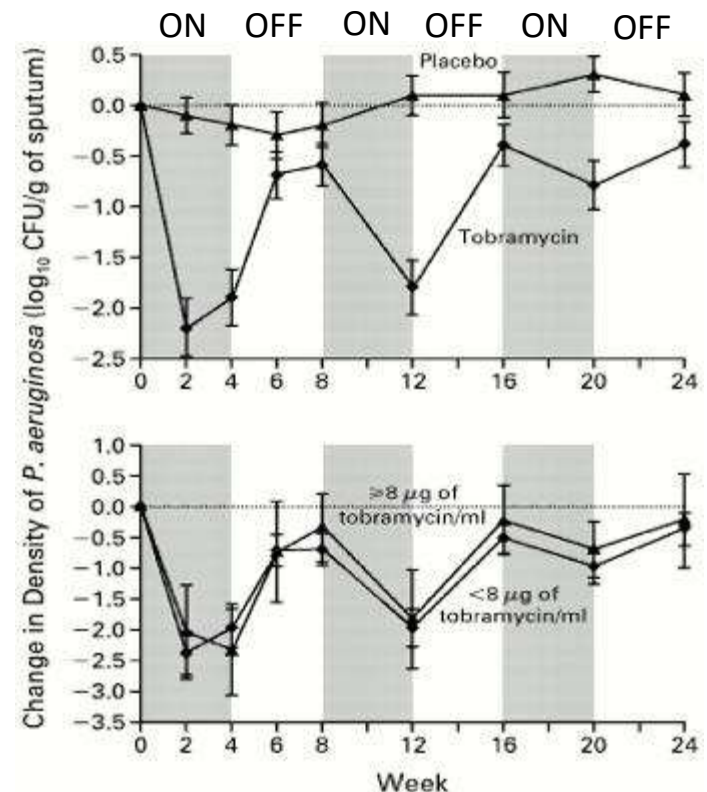
Levin-Reisman et al., *Science* 2017; 355:826-830

# Tobramycin Alone Ineffective

Modulates—not eradicates—  
*P. aeruginosa* infections

Resistance not responsible for  
▼ sputum CFU reduction over  
time

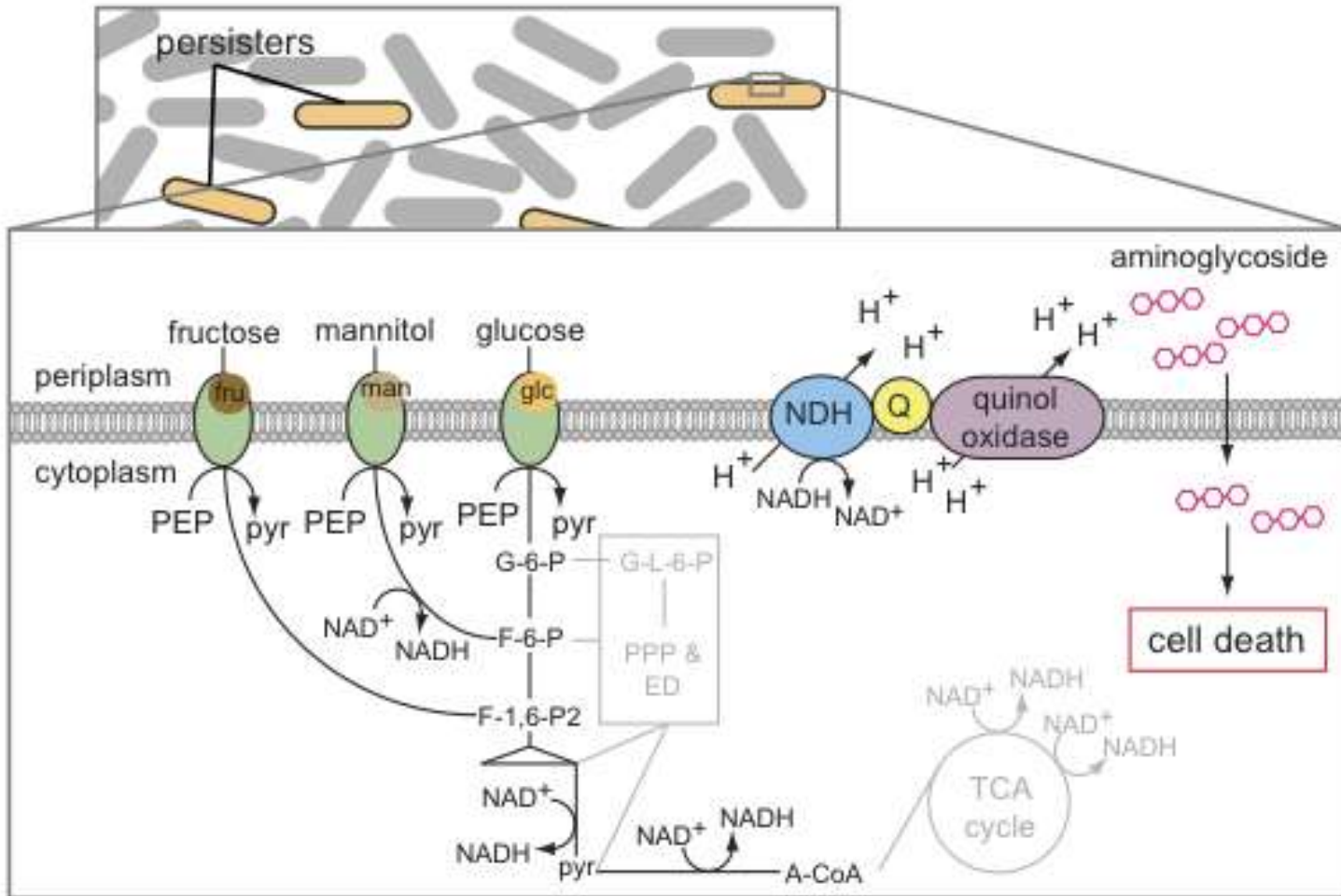
Clinical impact diminishes over  
time due in significant part to  
persisters



Ramsey et al., *NEJM* 1999; 340:23-30

Gibson et al., *Am J Respir Crit Care Med* 2003; 168:918-951

# Metabolite-Enabled Eradication of Bacterial Persisters is Proton Motive Force Mediated



Allison et al., *Nature* 2011; 473:216-220

# EBX-001 Executive Summary

## Significant Unmet Need:

- Vast majority of CF patients have recurrent *Pae* infections, primarily responsible for lung function decline & mortality
- Inhaled tobramycin is front-line therapy

## Target Product Profile:

- Tobramycin & potentiator (fixed ratio) for nebulized & dry powder inhalation
- Superior FEV1, CFU & frequency-of-exacerbation efficacy vs. tobramycin alone
- Potential to reduce therapy intensity

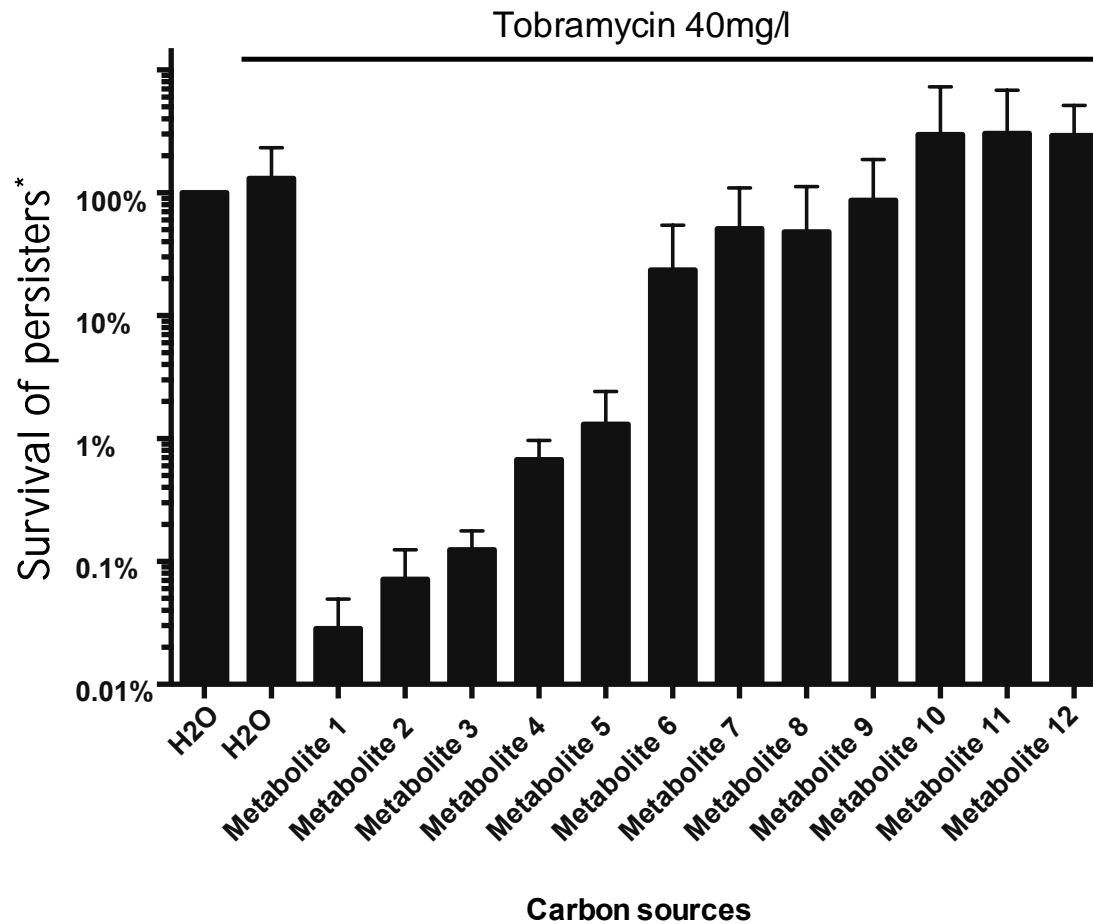
Lower Clinical/Regulatory Risk due to approved status of both tobramycin & potentiator

Patents cover metabolite-mediated potentiation & EBX-001 specifically



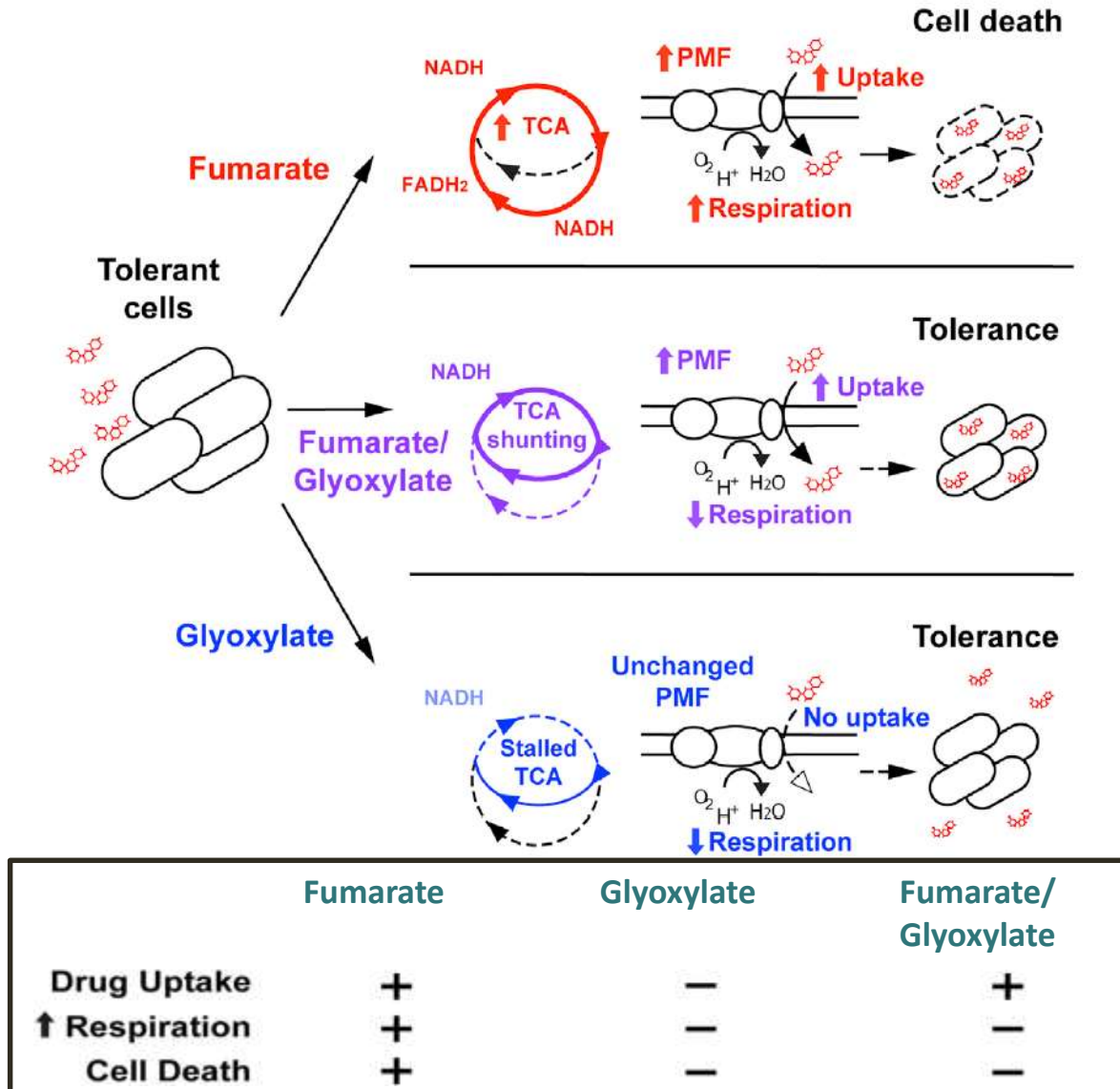


# Multiple Metabolites Screened to Yield Fumarate as Chosen Tobramycin Potentiator



\*Persisters selected by pretreatment with ofloxacin

# MOA of Fumarate: TCA Cycle & Respiratory Activity Required for Tobramycin Lethality



# EBX-001: Program Validation Thus Far

## Clear In vitro Efficacy

Dramatically enhanced killing of persisters across wide range of CF clinical isolates

## Robust Effect

1. In naïve biofilm models
2. Across relevant pH values
3. In artificial media and patient sputum
4. In presence of azithromycin
5. In mouse bronchitis model

## Indication Expansion

Similar potentiation observed in COPD clinical isolates

## Cytoprotective

Cytoprotective effect on human airway epithelial cells—potential to reduce airway inflammation

M. Koeva, A.D. Gutu, W. Hebert, J.D. Wager, L. Yonker, G.A. O'Toole, F.M. Ausubel, S.M. Moskowitz, D. Joseph-McCarthy, *Antimicrob Agents Chemother* 2017; 61: e00987-17.





# Vast Majority of Tested Strains Susceptible

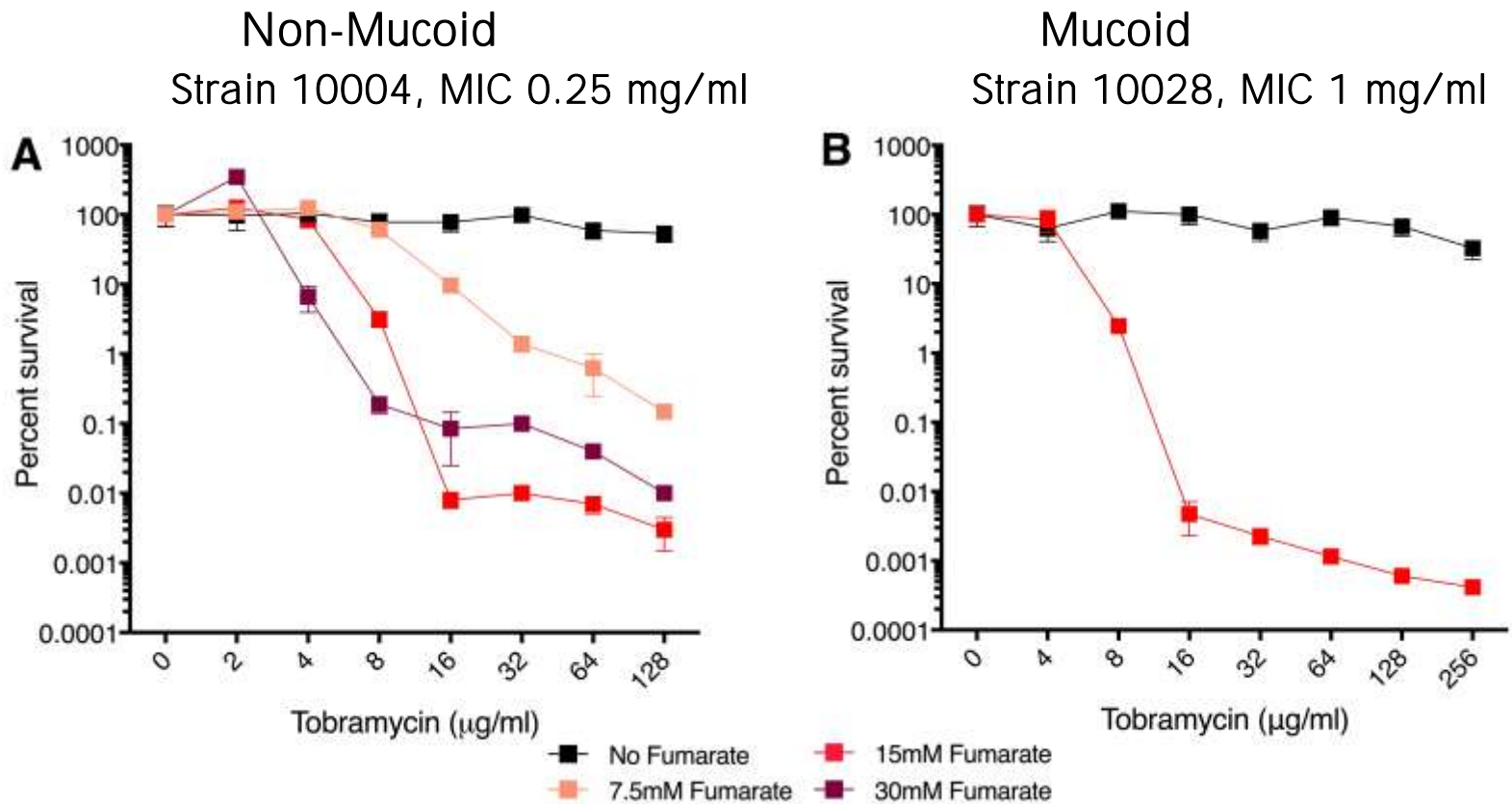
Strain #	Mucoid	MIC (CLSI)	Potential
10028	yes	1	5 logs
10048	yes	0.25	4.5 logs
10005	yes	0.25	5 logs
14003	yes	0.5	6 logs
10140	yes	0.5	5 logs
10063 small	yes	0.5	4.5 logs
10063 big	no	0.25	4.5 logs
10013	no	0.25	4 logs
10019	no	0.25	5 logs
10002	no	0.25	4 logs
10001	no	0.25	5 logs
10008	no	0.25	4.5 logs
10004	no	0.25	4.5 logs
10045	no	0.25	3 logs
10054	no	0.5	5 logs
14001	no	0.5	3.5 logs
10077	yes	0.25	no
10020	no	0.5	no

90%

of tobramycin-sensitive  
clinical isolates tested  
show significant  
potentiation effect

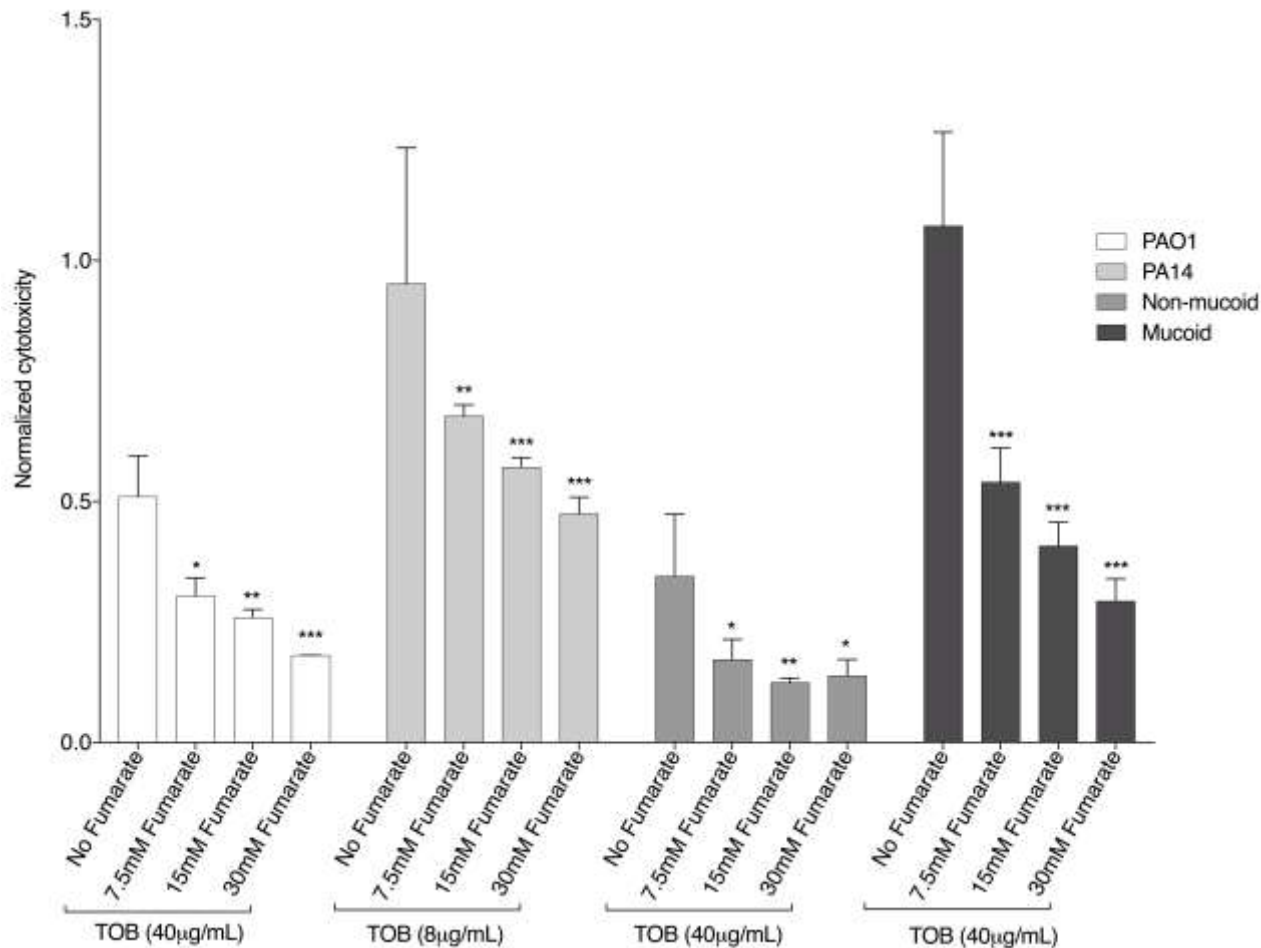


# Potential Effect in Non-Mucoid & Mucoid CF Strains



- Over 5 orders of magnitude of potentiation
- Maintained at high TOB concs (tested to 1024  $\mu\text{g/ml}$ )

# Potentiator Shows Cytoprotective Effect on Human Airway Epithelial Cells




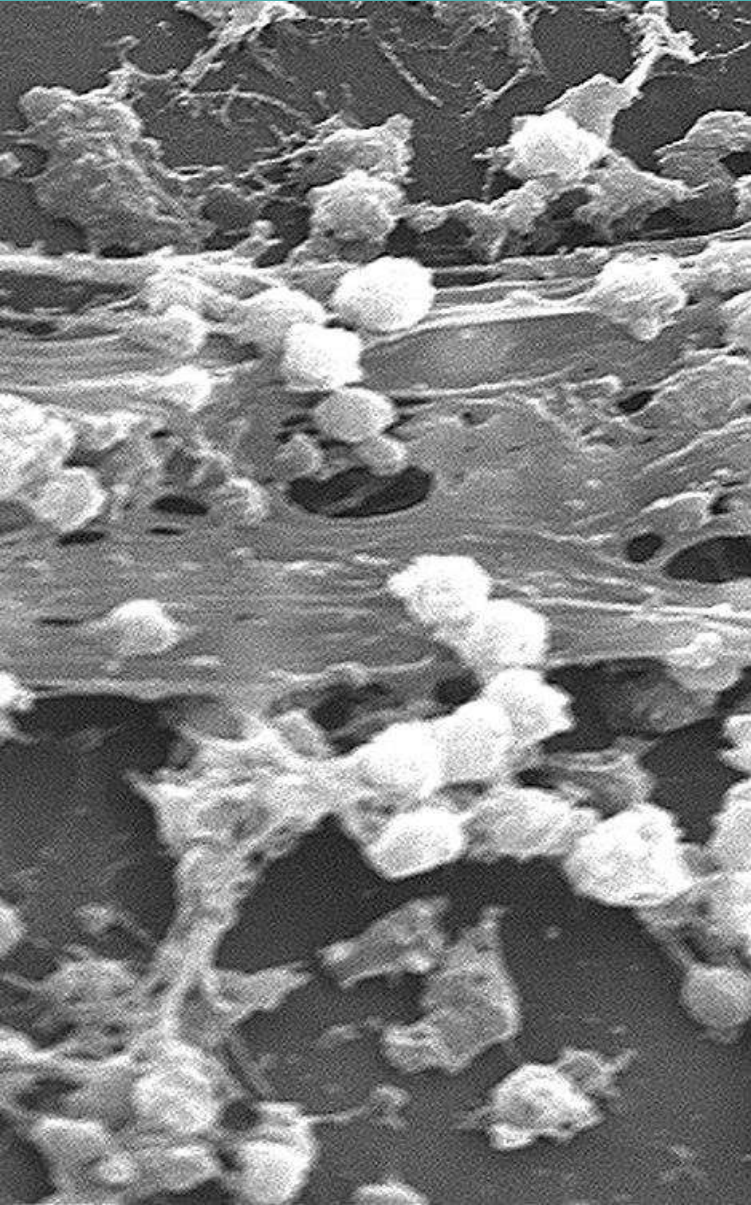
Potentiator

*Pseudomonas*  
cytotoxicity

\*Cytotoxicity in LDH assay normalized to that of the human airway epithelial cells in response to PAO1 *P. aeruginosa* untreated with TOB or fumarate



# Biofilms Enhance CF Infections



Disease Severity



Mucoid  
Phenotypes



Biofilms



Persisters

*Pritt et al., Am J Clin Pathol, 2007.*

*Lebeaux et al., Microbiol Mol Biol Rev, 2014.*

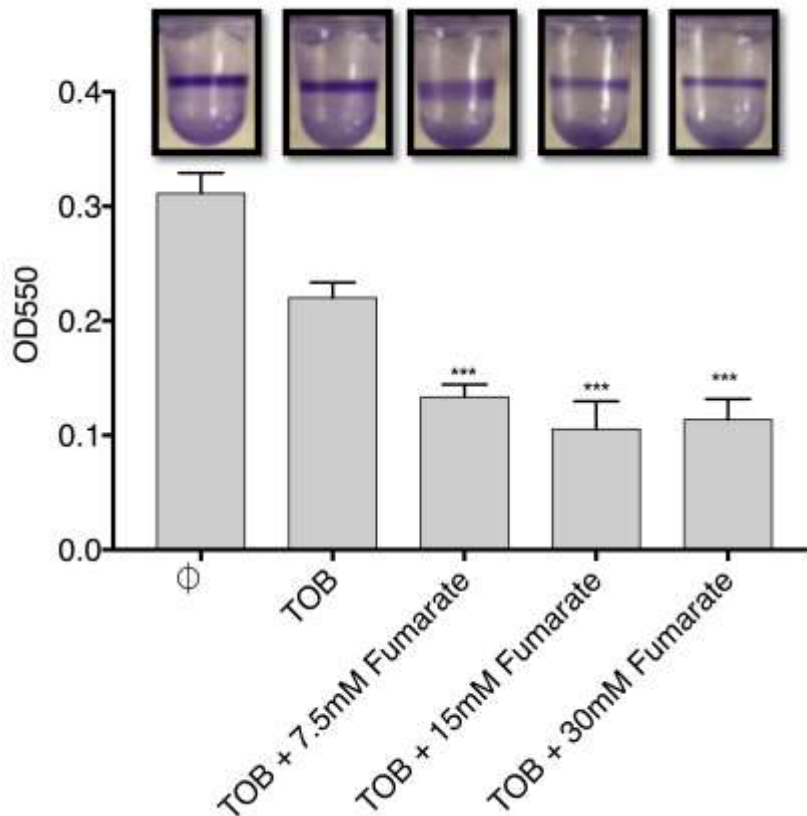


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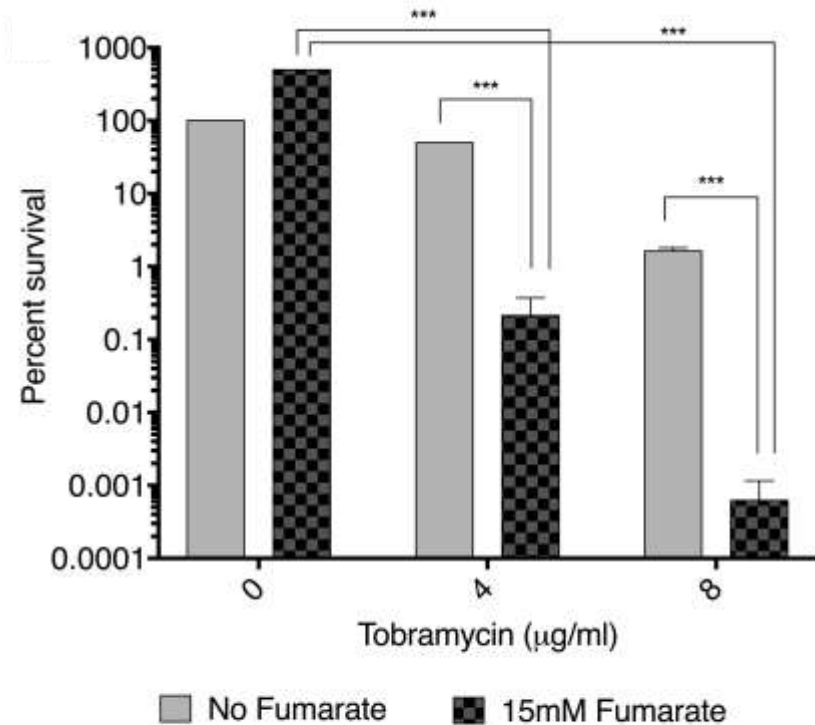
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# Potential Observed in Two Different Biofilm Assays

## 96-well plate biofilm assay



## Colony biofilm assay



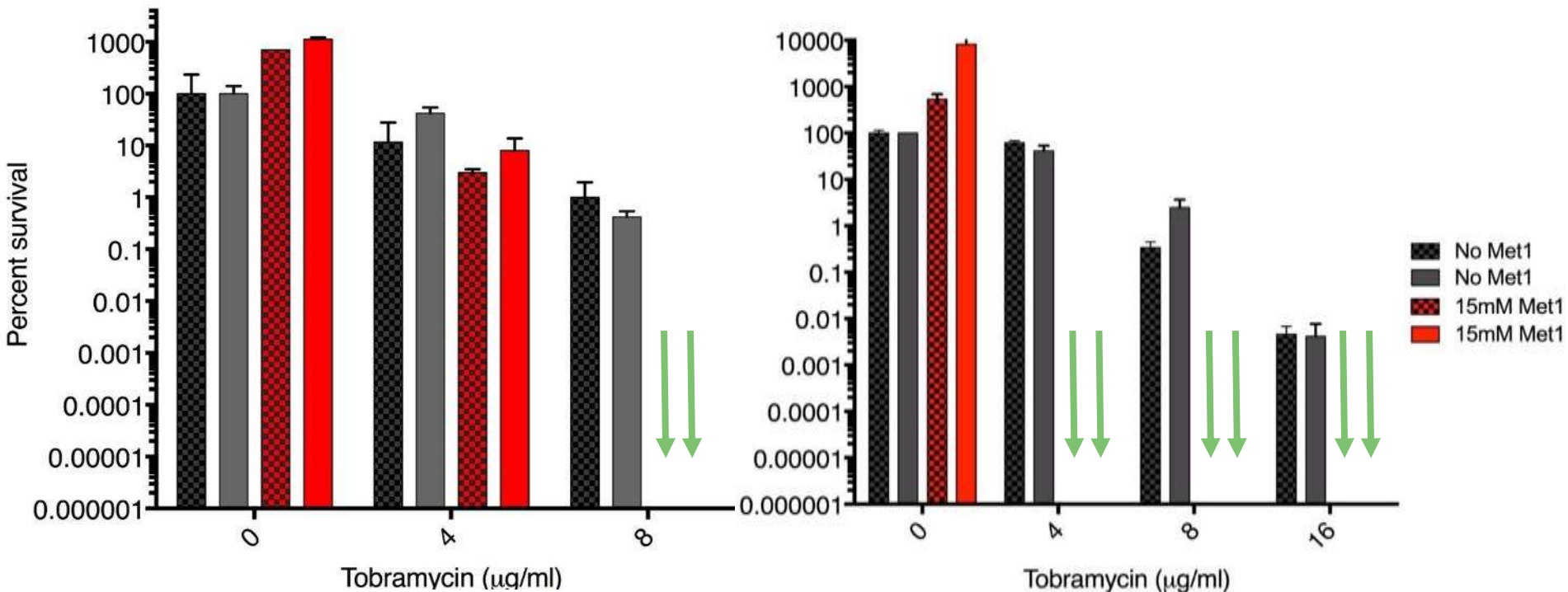
PA14 strain of *P. aeruginosa* tested



# Eradication of *P. aeruginosa* in Colony Biofilms From CF Clinical Isolates

## Non-mucoid strain 10004

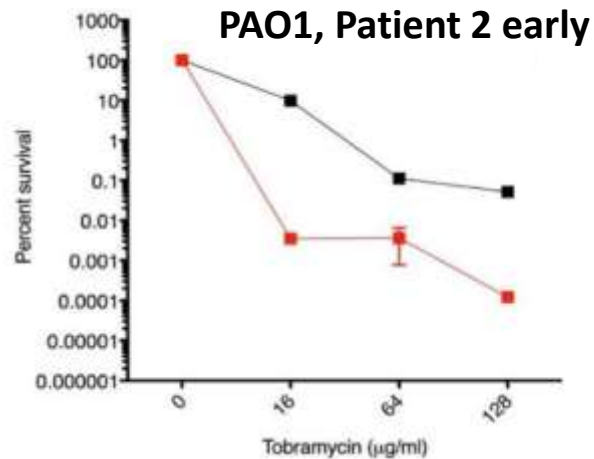
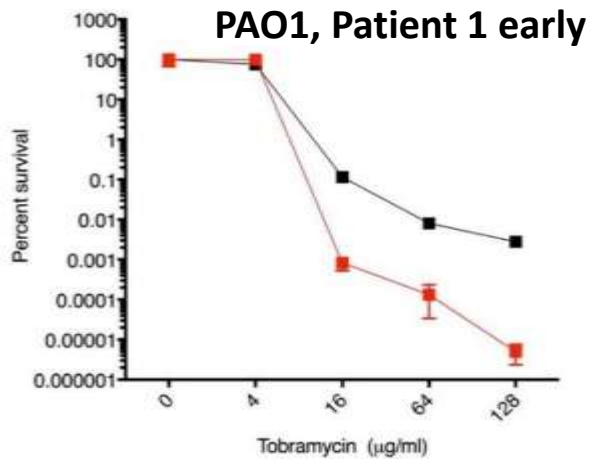
## Mucoid strain 10005



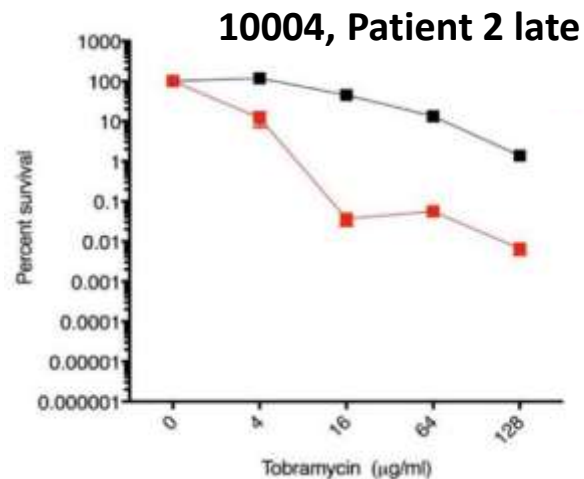
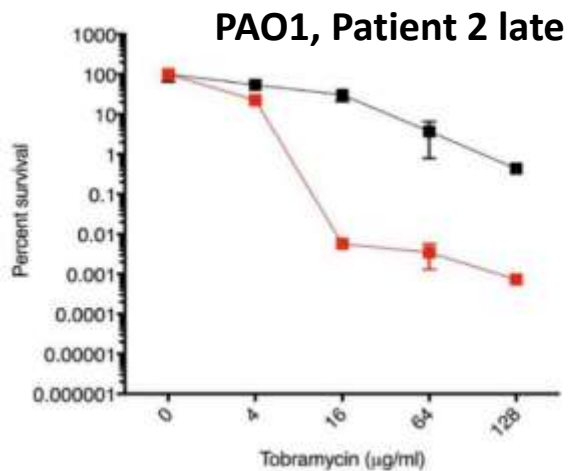
Arrows indicate complete eradication; No colonies



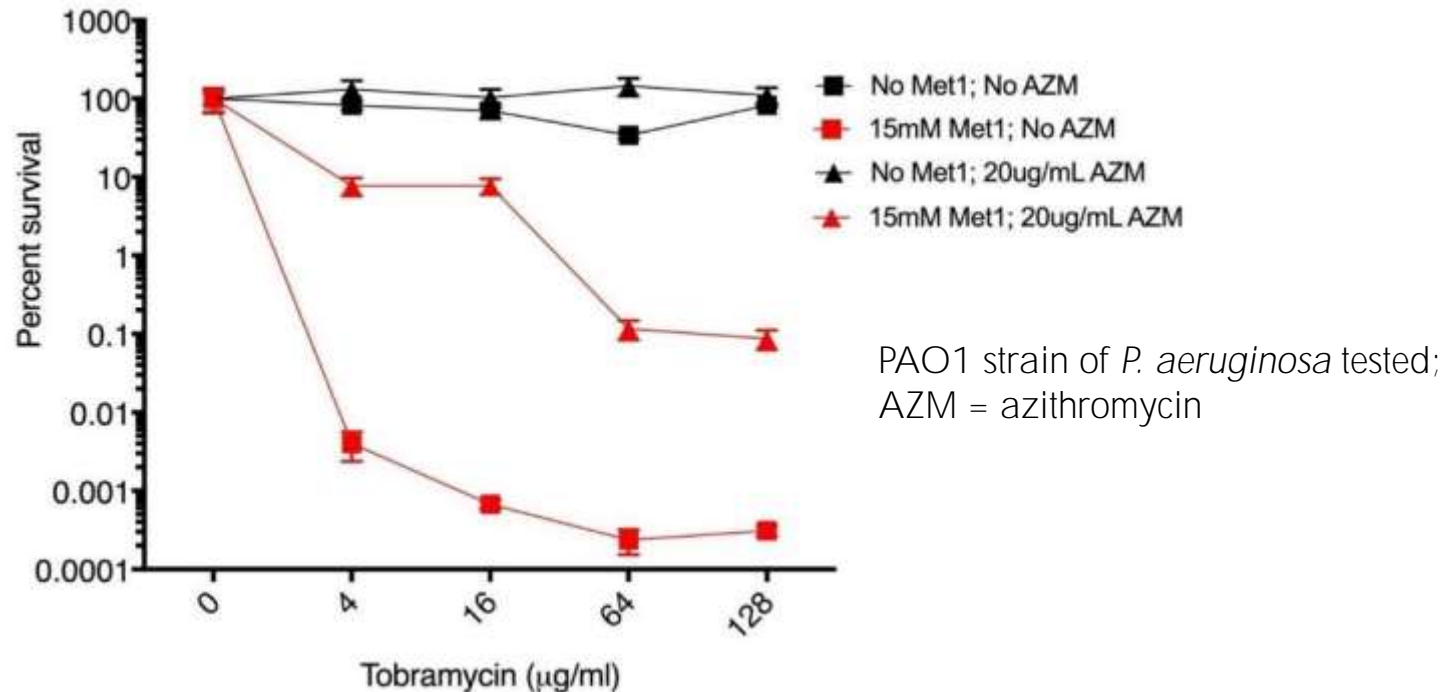
# 3 logs of Potentiation Observed In Presence of CF Sputum



■ No Met1  
■ 15mM Met1

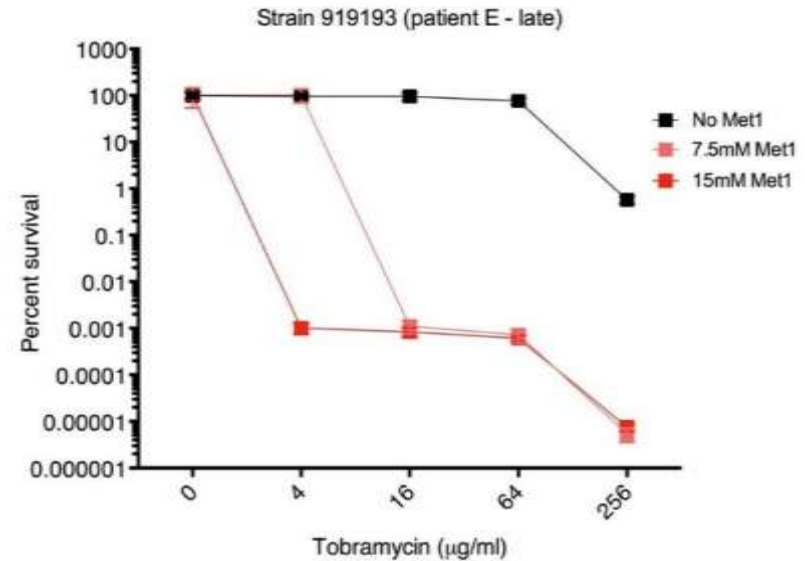
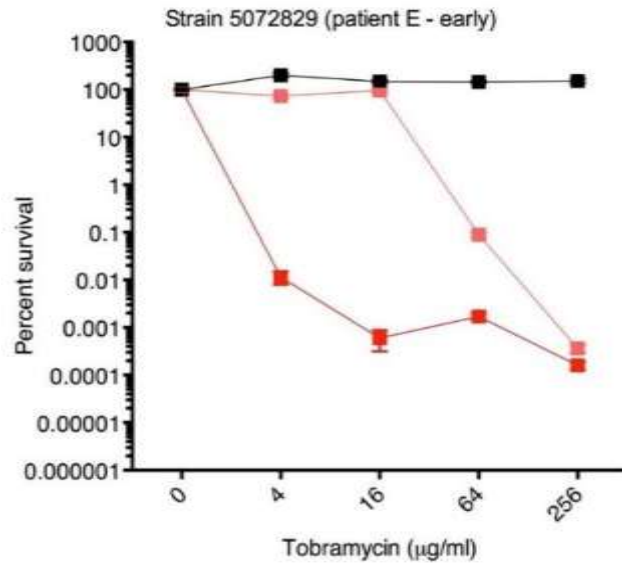


# >3 Logs of Tobramycin Potentiation In Presence of Azithromycin



- Azithromycin may antagonize the effect of tobramycin clinically
  - Nick et al., AnnalsATS, 2014
- “Among macrolides, azithromycin, to date, has been the most widely used for its potent anti-inflammatory effects ...”
  - Yousef & Jaffe, Paediatric Respiratory Reviews, 2010

# Potential Effect in COPD *P. aeruginosa* Strains

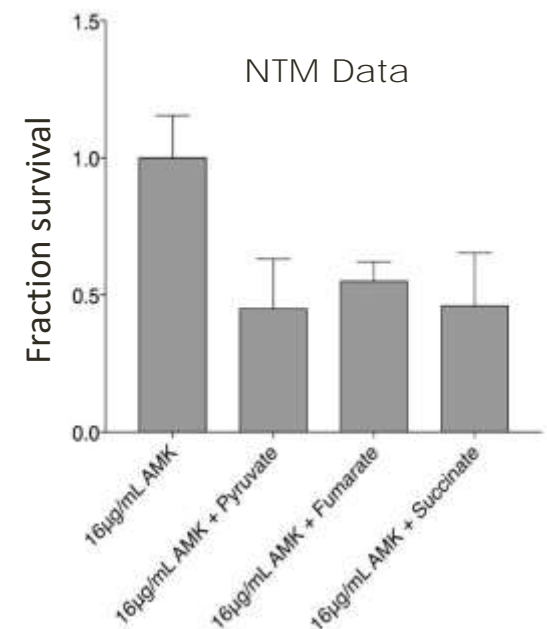
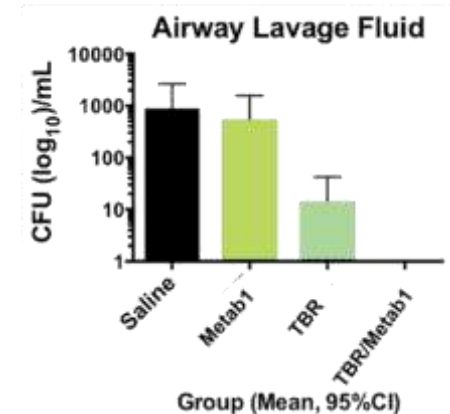


Strain	Year	Patient	E-test MIC	Broth MIC	Potential
16004728	2013	A (early)	1	-	5 logs
542368	2012	C (early)	0.38	0.125	3.5 logs
82346	2013	C (late)	0.064	0.125	3.5 logs
5054382	2009	D (early)	0.38	-	4 logs
5072829	2010	E (early)	1	0.25	5 logs
919193	2013	E (late)	0.016	0.25	5 logs



# Immediate Next Steps

- Confirm preliminary fibrin-plug mouse model results showing EBX-001 clears the infection and explore anti-inflammatory effect
  - Utilize agar bead model in collaboration with Tracey Bonfield, Case Western Reserve
- Pre-formulation and initial PK in rats to estimate ratio of dose to lung conc.
- Partnership with PARI to co-develop EBX-001 for use with eFlow
  - Formulation/device development
- Extension of the concept to NTM via EBX-002
  - Collaboration with Luiz Bermudez, Oregon State



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