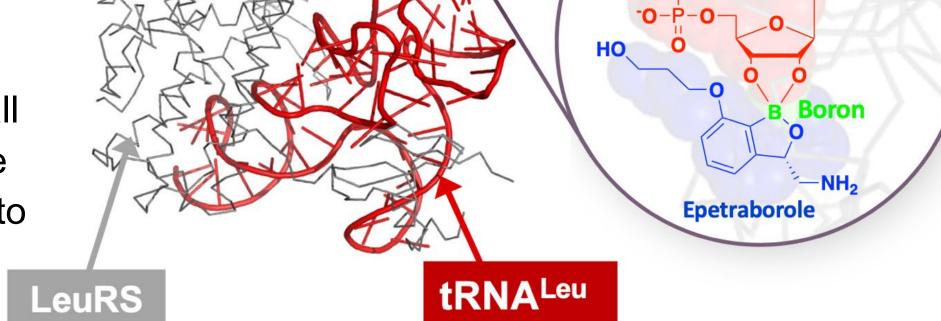
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# Epetraborole, a Potential Oral Agent for Mycobacterium abscessus Lung Disease

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isolates of Mycobacterium fortuitum were obtained from Barbara Brown-Elliott, Mycobacteria/Nocardia Research Laboratory, University of Texas Health Science Center at Tyler, TX; the Syracuse Veterans Administration Medical Center, Syracuse NY; SUNY Upstate Medical Center, Syracuse, NY; and Cincinnati Children's Hospital, Cincinnati, OH. Stocks of isolates were prepared and frozen at -80°C. Fresh cultures of each isolate (<1 week) were grown on Mueller-Hinton agar for use in each experiment. TIG MIC Modal 8 4 8 MIC sol 8 4 MIC sol 8 4 MIC sol 8 MIC Modal 8 4 MIC sol 8 MIC Modal 8 MIC sol 8 MIC Modal 8 MIC sol 8 MIC Modal 8 MIC sol 8 MIC Modal 8 MIC sol 8 MIC	INTRODUCTION					R	ESULT	S												
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9 and control   9 and control<	tuberculous mycobacteria (NTM). The macrolides, which are the backbone for most slow-																			
Highlights the need for more effective thirriples.   EBO   Mic Madel   0.06   0.06   0.06   0.06   0.06     The 3-aminomethyl benzosaborole (EBC). NN3565,   GSK2251052) is an orally biosvaliable   Mic Manage   0.06<	inducible resistance observed with <i>Mycobacterium abscessus</i> . The lack of effective treatment options for <i>M. abscessus</i> lung disease is reflected in its high all-cause mortality (44.8%), which						+		MC2	MC5	MC5	E B B	BB	BB	CUG	CUG	MC3			
Bit Bulk and relation table election into the election into all election into into the election into all election into into the election into all electin all election into all election into all ele			MIC Range	0.03-0.125	0.06-0.125	0.06	0	643 1997	643	806	605	Ñ	7	õ	A3	A4	044			
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Belatatorolog (EBO, MASSe). CLR INC and CCR			MIC <sub>50</sub>	0.06	0.06	0.06	AMK	I.	NE	I	I	I	I	I	I	I	I.			
CSK2251052) is an orally bioavailable inhibitor of the essential bacterial protein synthesis enzyme, leucyl-RNA synthesis enzyme,	epetraborole (EBO; AN3365,		MIC <sub>90</sub>	0.06	0.06	0.06	CLR		NE											
AMK   Micl work   16   16   16   16   2     protein synthesis enzyme, lawcy-HRNA synthesia elucy. BEO traps the terminal nucleolide of IRNA <sup>m</sup> in the editing site of LeuRS preventing IRNA <sup>tm</sup> from being aminoacylated; trus, leading to inhibition of protein synthesis. In addition to this novel mechanism of action, EBO is a small polar molecule with extensive tissue distribution and good penetration into alveolar macrophages'.   Micl work   16   16   1	GSK2251052) is an orally bioavailable		MIC Range	4 - >64	16 - 64	1 - 4			I				1	1	1					
product synthesis in Leading to inhibition of protein sing animoscylated; thus, leading to inhibition of protein addition to this novel mechanism of action, EBO is a small polar molecule with extensive tissue alveolar macrophages! <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b> <b>Example</b>	inhibitor of the essential bacterial	AMK	MIC Modal	16	16	2				I					1		_			
MIC Range   0.03 - >64   0.06 - 0.25   0.25 - 16     MIC Range   0.03 - >64   0.06 - 0.25   0.25 - 16     MIC Range   0.025   0.125   8     UP Relation of this novel mechanism of action, EBO is a small polar molecule with extensive tissue distribution and good penetration into avecolar macrophages <sup>1</sup> .   MIC Range   0.03 - >64   0.06 - 0.25   0.25 - 16     MIC Range   0.125 - 0.125   8   MIC Range   0.03 - 20 - 25   16     MIC Range   0.125 - 0.25   10   11 - 1   1	synthetase (LeuRS). EBO traps the terminal nucleotide of tRNA <sup>Leu</sup> in the editing site of LeuRS preventing tRNA <sup>Leu</sup> from being aminoacylated; thus, leading to inhibition of protein synthesis. In addition to this novel mechanism of action, EBO is a small polar molecule with extensive tissue distribution and good penetration into alveolar macrophages <sup>1</sup> .		MIC <sub>50</sub>	16	16	2	CFX													
celling site of LeuRS preventing tRNA-tew from being aminoacylated; thus, leading to inhibition of protein synthesis. In addition to this novel mechanism of action, EBO is a small polar molecule with extensive tissue distribution and good penetration into alveolar macrophages!			30			4	LZD	1		I		I		I			l I			
editing site of LeuRS preventing tRNAI <sup>eu</sup> from being aminoacylated; thus, leading to inhibition of protein synthesis. In addition to this novel mechanism of action, EBO is a small polar molecule with extensive tissue distribution and good penetration into alveolar macrophages! <b>EVENDES</b> <b>IDENTIFY of EAS Health Science Center at NIC Modal</b> <b>IDENTIFY St. the Synacuse NY; and Cincinnatic Children's Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Center, Syracuse NY; and Cincinnatic Children's Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Center, Syracuse NY; and Cincinnatic Children's Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Center, Syracuse NY; and Cincinnatic Children's Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Center, Syracuse NY; and Cincinnatic Children's Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Center, Syracuse NY; and Cincinnatic Children's Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Center, Syracuse NY; SUNY Upstate Mick Modal Center, Syracuse NY; and Cincinnatic Children's Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Center, Syracuse NY; and Cincinnatic Children's Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Center, Syracuse NY; and Cincinnatic Children's Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Conter, Syracuse NY; and Cincinnatic Children's Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Conter, Syracuse NY; and Cincinnatic Children's Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Conter, Syracuse NY; and Cincinnatic Children's Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Conter, Syracuse NY; and Cincinnatic Children's Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Conter, Syracuse NY; and Cincinnatic Children's Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Conter, Syracuse NY; Conter At Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Conter, Syracuse NY; Conter At Hospital Center, Syracuse NY; SUNY Upstate Mick Modal Conter, Syracuse NY; Co</b>						0. 25 - 16	TIG	I.					I	I	I		1			
tRNAle from being aminoacylated;   0.23   0.125   8     thus, leading to inhibition of protein synthesis. In addition to this novel mechanism of action, EBO is a small polar molecule with extensive tissue distribution and good penetration into alveolar macrophages <sup>1</sup> .   Imilesa Micesa   0.25   0.125   0   0   125   0     Micesa   0.125   0.125   0   0.125   0   0   125   0   0   125   0   0   0   0   0   0   0   0   125   0   0   0   0						8	IMP	1	1		1		I	1	I	1				
Index, reading to minimum of protein synthesis. In addition to this novel mechanism of action, EBO is a small polar molecule with extensive tissue distribution and good penetration into- alveolar macrophages <sup>1</sup> .   Image: 0.125 - 0.5   0.125 - 0.5   0.125 - 0.5   0.125 - 0.5   0.125 - 0.5     Image: 0.125 - 0.5   0.25   0.25   0.25   0.25   0.25   0.25   0.25   0.05   0.6   0.05   0.05   0.05   0.05   0.05   0.05   0.06   0.125 - 0.5   0.06   0.05   0.05   0.05   0.05   0.06   0.125 - 0.5   0.06   0.05   0.05   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.06   0.06   0.06   0.0125, and   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.05   0.06   0.05			50	0.25		8			, ,	• • •	•	I	י איוי ויי	1 1 · · · ·	•	I	1			
Synthesis. In addition to EBo yas small polar molecule with extensive tissue distribution and good penetration into al good penetration into alloging in the penetration isolates of Mycobacterium chelonee, and 10 M contal interval into alloging in the penetration isolate into the penetration isolates of Mycobacterium isolates and Mycobacterium isolates are haborated. The penetratio			50	2			I = Indimerent, N = - no ending point to due to non-susceptibility to combination drug													
Interchains of a statility operation of seasing operations of the season operation of the season operation operation operation operation operations operating the prestreform operations operations operations oper		CFZ						isolates were tested for their activity. The EBO MIC range for <i>M. abscessus</i> , <i>M. chelonae</i> , and <i>M. fortuitum</i> was 0.03 - 0.125,												
MC and good penetration into distribution and good penetration into distribution and good penetration into alveolar macrophages!.   MC ange   1   0.5   0.5   M. abscessus, M. chelonae, and M. fortuitum was 0.03 - 0.125, Model MIC sole of Mic sole																				
Mic Range   2-64   >256   8-64   0.06 - 0.125, and 0.06 mg/L, respectively. Furthermore, the EBO     alveolar macrophages1.   Mic Modal   16   >256   32   Modal MIC, MIC <sub>50</sub> , and MIC <sub>90</sub> for the entire MABS panel of 39 isolates of M. abscessus, 10 isolates of Myocbacterium chelonae, and 10 isolates of Myocbacterium fortuitum were obtained from Barbara Brown-Elliott, isolates of Mycobacteria/Nocardia Research Laboratory, University of Texas Health Science Center at Mic <sub>90</sub> 8   4   8     Mic Modal   8   4   8   4   8   4   8   4   8   4   8   4   8   4   8   4   8   4   8   8   4   8   8   4   8   9   9   9   9   9   9   9   9			00	0.5																
Mice   Mic Modal   16   >256   32   Modal MIC, MIC <sub>50</sub> , and MIC <sub>90</sub> for the entire MABS panel of 39 isolates was 0.06, 0.06, and 0.06 mg/L, respectively (Table 1).     Mice   Mice   0.5 ->64   1-8   2-8     Mice   Mice   8   4   8   9   9   9   9   90   90   100			50																	
Mic   Subject		CFX																		
METHODS   Mics   32   >256   32   The activity of EBO was not affected by macrolide, aminoglycoside, or LZD resistance.     Isolates: A total of 39 isolates of <i>M. abscessus</i> , 10 isolates of <i>Myocbacterium chelonae</i> , and 10 isolates of <i>Mycobacterium fortuitum</i> were obtained from Barbara Brown-Elliott, Mycobacteria/Nocardia Research Laboratory, University of Texas Health Science Center at Tyler, TX; the Syracuse Veterans Administration Medical Center, Syracuse NY; SUNY Upstate Mics   Mic Range   0.5->64   1-8   2-8   The activity of EBO was not affected by macrolide, aminoglycoside, or LZD resistance.     MIC Modal   8   4   8   4   8   4   8   assay (Table 2). EBO was not antagonized by any of these drugs with any of the <i>M. abscessus</i> strains we tested in the checkerboard assay (Table 2). EBO activity was found to be indifferent in combination with AMK, CLR, CFZ, CFX, LZD, TIG, or IMP.     TIG   MiC Modal   0.125, 0.5   0.06   0.015, 0.03   onton with AMK, CLR, CFZ, CFX, LZD, TIG, or IMP.									00		00				-					
Isolates: A total of 39 isolates of M. abscessus, 10 isolates of Myocbacterium chelonae, and 10 isolates of Myocbacterium fortuitum were obtained from Barbara Brown-Elliott, Mycobacteria/Nocardia Research Laboratory, University of Texas Health Science Center at Tyler, TX; the Syracuse Veterans Administration Medical Center, Syracuse NY; SUNY Upstate MIC <sub>90</sub> MIC Range   0.5->64   1-8   2-8   or LZD resistance.     MIC Modal   8   4   8   4   8   4   8   any of the M. abscessus strains we tested in the checkerboard assay (Table 2). EBO activity was found to be indifferent in the checkerboard assay (Table 2). EBO activity was found to be indifferent in combination with AMK, CLR, CFZ, CFX, LZD, TIG, or IMP.     TIG   MIC Modal   0.125   0.06   0.015   0.015   0.015			00										•	-	•		,			
Isolates of Mycobacterium fortuitum were obtained from Barbara Brown-Elliott, Mycobacteria/Nocardia Research Laboratory, University of Texas Health Science Center at Tyler, TX; the Syracuse Veterans Administration Medical Center, Syracuse NY; SUNY Upstate Medical Center, Syracuse, NY; and Cincinnati Children's Hospital, Cincinnati, OH. Stocks of isolates were prepared and frozen at -80°C. Fresh cultures of each isolate (<1 week) were grown on Mueller-Hinton agar for use in each experiment.   MIC Modal 8   4   8   1   The activity of EBO was not antagonized by any of these drugs with any of the <i>M. abscessus</i> strains we tested in the checkerboard assay (Table 2). EBO activity was found to be indifferent in combination with AMK, CLR, CFZ, CFX, LZD, TIG, or IMP.	<b>Isolates:</b> A total of 39 isolates of <i>M. abscessus</i> , 10 isolates of <i>Myocbacterium chelonae</i> , and 10 isolates of <i>Mycobacterium fortuitum</i> were obtained from Barbara Brown-Elliott, Mycobacteria/Nocardia Research Laboratory, University of Texas Health Science Center at		50				or LZD resistance.													
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Medical Center, Syracuse, NY; and Cincinnati Children's Hospital, Cincinnati, OH. Stocks of isolates were prepared and frozen at -80°C. Fresh cultures of each isolate (<1 week) were grown on Mueller-Hinton agar for use in each experiment. TIG MIC Modal 0.125, 0.5 0.06 0.015, 0.03 - 0.125 0.06 0.015, 0.03 - 0.015 0.015, 0.03 - 0.015, 0.			50	32	8	8	assay (	Table 2	2). EE	30 ac	ctivity	was f	ound	to be	indiffe	erent	in			
isolates were prepared and frozen at -80°C. Fresh cultures of each isolate (<1 week) were grown on Mueller-Hinton agar for use in each experiment. IIG MIC Modal 0.125, 0.5 0.06 0.015, 0.03 MIC 50 0.125 0.06 0.015			50		0 03 - 0 125	0 008 - 0 06	combinat	tion wit	h AMK	K, CLR	, CFZ,	CFX,	LZD, <sup>-</sup>	ГIG, or	IMP.					
grown on Mueller-Hinton agar for use in each experiment. $0.125$ $0.06$ $0.015$	isolates were prepared and frozen at -80°C. Fresh cultures of each isolate (<1 week) were	TIG																		
	grown on Mueller-Hinton agar for use in each experiment.			,																
	<b>Antibacterial susceptibility testing:</b> Initial tests of EBO with <i>Mycobacterium peregrinum</i> ATCC 700686 were performed in CAMHB to verify its activity, and a minimal inhibitory concentration		MIC <sub>90</sub>	0.5	0.06	0.03														



Antibacte 700686 were performed in CAMHB to verify its activity, and a minimal inhibitory concentration (MIC) value of 0.06 mg/L was obtained. This value is within the acceptable CLSI QC range of 0.03-0.12 mg/L. All RGM isolates were tested as described by CLSI using broth microdilution in CAMHB with 2-fold dilutions of antimicrobials. The MIC values were read after incubation at 30°C for 3-4 days, when sufficient growth was evident in the growth control wells. The breakpoints in the CLSI document M24S<sup>2</sup> were used to determine resistance, for example MIC values for amikacin (AMK) of  $\geq 64$  mg/L, clarithromycin (CLR) of  $\geq 8$  mg/L, and linezolid (LZD) of  $\geq$ 32 mg/L are deemed to be resistant.

Antibacterial synergy testing: The effects of combining EBO with AMK, CLR, clofazimine

## DISCUSSION

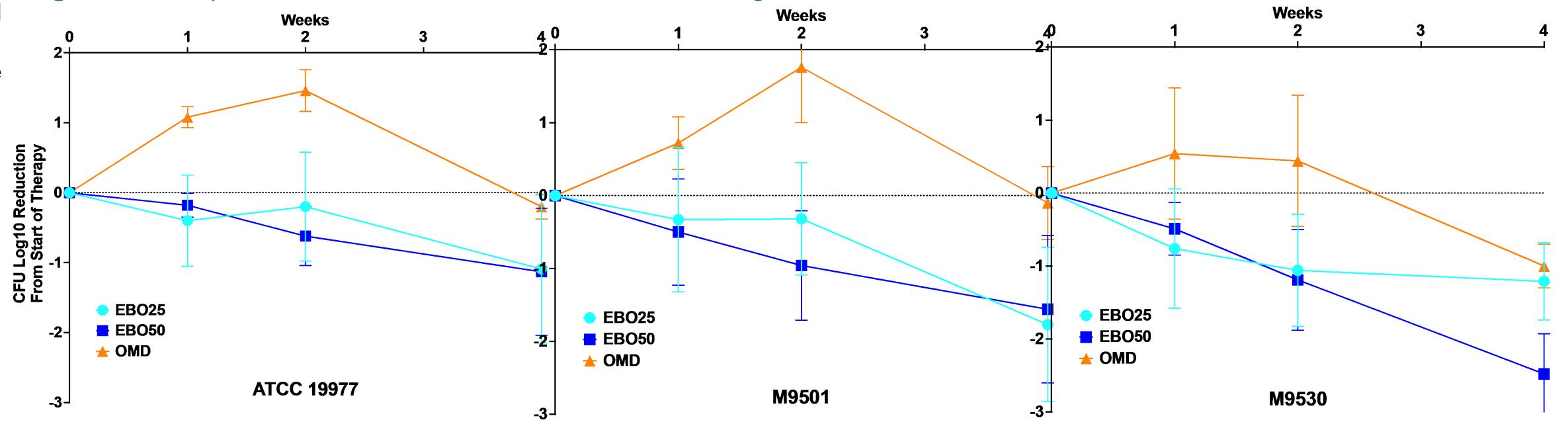
The need for new therapies for *M. abscessus* lung disease is highlighted by the limited choices of potent and effective oral therapies, which is made worse by the fact that mycobacterial diseases need to be treated with combination therapy. Herein, we confirm the previous observations of Nguyen et al.<sup>3</sup> that EBO has good in vitro potency against *M. abscessus* and unlike ganfeborole<sup>4</sup>, another LeuRS inhibitor, EBO has activity against other rapidly growing mycobacteria like *M. fortuitum*. In addition, EBO is not antagonistic to the commonly used drugs for *M. abscessus* lung disease. Since omadacycline (OMD) demonstrated efficacy in a phase 2b study of *M. abscessus* lung disease with higher rates of negative sputum cultures for *M. abscessus* (56.4% vs 29.2%, p=0.035) at Day 84 compared with placebo<sup>5</sup>, we compared the reported murine efficacy of EBO<sup>6</sup> and OMD<sup>7</sup> in the dexamethasone treated C3HeB/FeJ lung infection model of M. abscessus. The murine efficacy of EBO compared very well to OMD suggesting that if this mouse model data translated to humans EBO could potentially be a new oral agent for *M. abscessus* lung disease.

(CFZ), cefoxitin (CFX), LZD, tigecycline (TIG), or imipenem (IMP) were evaluated in 10 MABS isolates. Synergy, additive effects, indifference or antagonism was characterized in the checkerboard assay using EUCAST criteria. Synergistic or antagonistic activity was determined using the sum of the fractional inhibitory concentration ( $\Sigma$ FIC) index. The FIC index is calculated as the sum of FIC A + FIC B, where FIC A is the MIC of drug A in the combination of drugs A and B divided by the MIC of drug A alone, plus the MIC of drug B in the combination of drugs A and B divided by the MIC of drug B alone. A combination of drugs is considered synergistic when the FIC is ≤0.5, additive when the FIC is >0.5 to 1, indifferent when the FIC is >1 to 2, and antagonistic when the FIC is >2 using EUCAST criteria.

# CONCLUSIONS

- EBO has potent in vitro activity against *M. abscessus* and other rapidly growing mycobacteria
- The activity of EBO was not affected by macrolide, aminoglycoside, or LZD resistance
- No antagonisms were observed with any *M. abscessus* strain tested
- All EBO interactions were indifferent for all drugs

### **Figure 1.** Comparison of EBO<sup>6</sup> and OMD<sup>7</sup> in a Mouse Lung Infection Model of *M. abscessus*



The AUC<sub>0-24</sub> of EBO25 and EBO50 in C3HeB/FeJ mice are equivalent to human EBO oral exposures of 250 and 500 mg q24h. The EBO MIC values for *M. abscessus* ATCC 19977, M9501, and M9530 were 0.0625 mg/L compared with 0.375, 0.25, and 0.125 mg/L for OMD.

#### ACKNOWLEDGEMENTS

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# These data support further investigation of EBO as a potential therapy for *M. abscessus* lung disease

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