Epetraborole:
A Novel Antibiotic for NTM Lung Disease & Melioidosis

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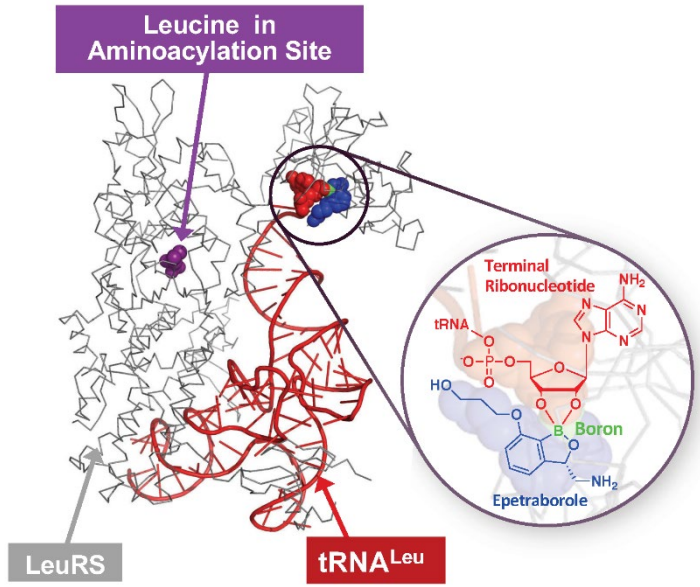
A new approach to global health

www.an2therapeutics.com

Epetraborole

Overview

- **Novel mechanism of action (MOA)**[1]
- **In vitro activity against nontuberculous mycobacteria (NTM) and *Burkholderia pseudomallei***
- **Oral formulation in late-stage development for NTM lung disease**
  - QIDP, Fast Track, and Orphan Drug designations granted in U.S.
  - Superior microbiological efficacy when combined with SOC compared to SOC alone in preclinical NTM animal models [2]
  - Multiple Phase 1 studies support well-tolerated dose (500 mg QD) with high probability of target attainment for *Mycobacterium avium* complex (MAC) [3,4]
  - Phase 2/3 pivotal trial in treatment-refractory MAC lung disease currently enrolling (**ClinicalTrials.gov NCT05327803**)
  - Nonclinical development underway to support clinical development for *M. abscessus* lung disease
- **IV formulation in early-stage development for melioidosis**
  - Awarded NIAID contract in 2022 to support pre-Phase 3 studies
  - Observational study in Thailand & Laos currently enrolling



MOA: Epetraborole inhibits leucyl-tRNA synthetase (LeuRS) by binding to the terminal adenosine ribose of $tRNA^{Leu}$ in the editing site, blocking protein synthesis.[1]

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QIDP = Qualified infectious disease product; SOC = Standard-of-care. [1] Hernandez V, et al. *Antimicrob Agents Chemother* 2013;57:1394. [2] De K, et al. IDWeek 2022 Poster. [3] Bhavnani SM, et al. IDWeek 2022 Poster. [4] Eckburg PB, et al. IDWeek 2022 Poster & Presentation.

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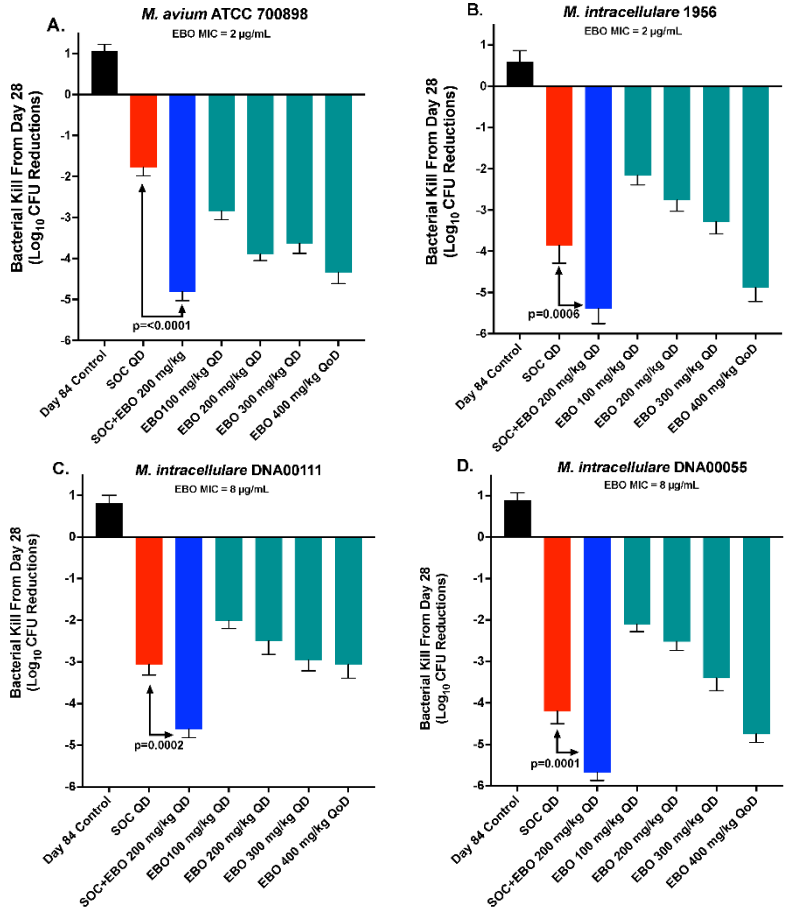
Potent In Vitro & In Vivo Activity vs. MAC

- **110 respiratory MAC isolates from Japan**
  - MIC range 0.25–16 µg/ml; MIC_{90} = 4 µg/ml
  - Macrolide resistance did not impact EBO activity
  - MIC distributions similar to those associated with isolates collected from the U.S.[1]
  - See **Poster #2135** (Sat, Oct 14, 12:15-1:30PM)

Antimicrobial	MIC (µg/mL)		
	MIC Range	MIC_{50}	MIC_{90}
Epetraborole	**0.25 – 16**	**2**	**4**
Clarithromycin	0.125 – >32	1	4
Amikacin	2 – 32	8	16
Ethambutol	2 – >32	4	16
Rifabutin	≤0.03 – 2	0.06	0.25

- **Superior CFU reductions with EBO + SOC vs. SOC alone** with 4 MAC isolates in a chronic mouse model of MAC lung disease [2]



CFU = Colony-forming units; MAC = *Mycobacterium avium* complex; MIC = Minimum inhibitory concentration; SOC = Standard-of-care (clarithromycin, ethambutol, and rifabutin). [1] DeStefano, MS, et al. IDWeek 2022 Poster. [2] De K, et al. IDWeek 2022 Poster.

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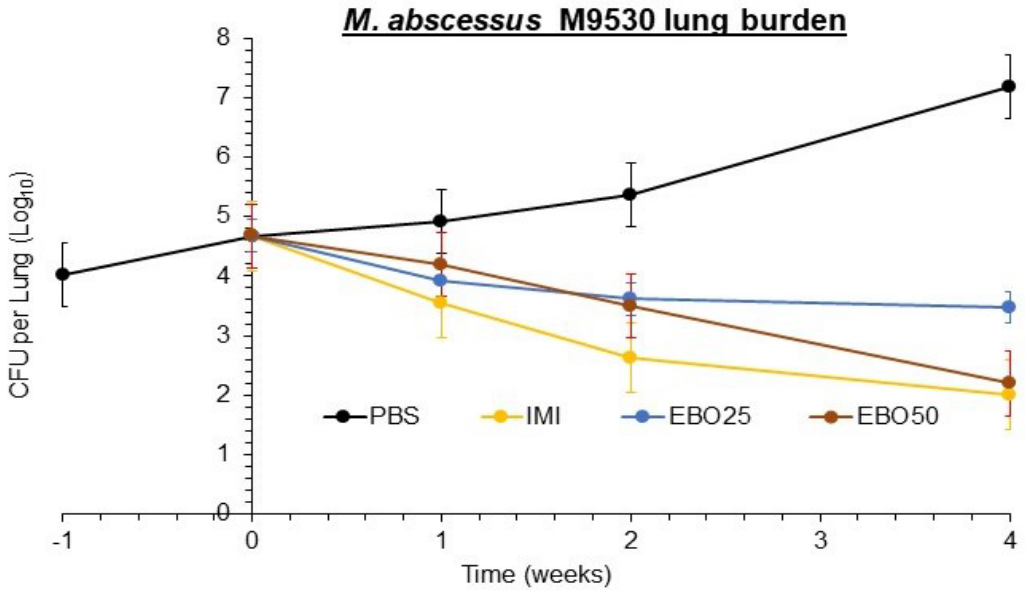
Potent In Vitro & In Vivo Activity vs. M. abscessus (Mab)

- **147 respiratory Mab isolates from U.S. & Europe**
  - MIC range 0.03–0.25 µg/ml; MIC_{90} = 0.12 µg/ml
  - Macrolide resistance, amikacin resistance, and morphology did not impact EBO activity
  - See **Oral #2064** (Sat, Oct 14, 10:54-11:06 AM)

Antimicrobial	MIC (µg/mL) MIC range	MIC_{50}	MIC_{90}
Epetraborole	**0.03 - 0.25**	**0.06**	**0.125**
Clarithromycin	≤0.25 - >32	>32	>32
Amikacin	4 - 64	16	64
Imipenem	≤1 - >32	8	32
Linezolid	≤0.5 - >16	16	>16
Moxifloxacin	≤0.5 - >4	4	>4
Cefoxitin	4 -128	32	64
Doxycycline	0.25 - >4	>4	>4
Tobramycin	4 - >8	>8	>8
Clofazimine	≤0.25 - 1	0.5	1
Minocycline	≤0.125 - >8	>8	>8
Tigecycline	0.25 -1	0.25	1
Rifabutin	0.5 - >4	>4	>4
Ethambutol	8 - >32	>32	>32

- **Similar CFU reductions vs. imipenem in a chronic model of Mab lung disease (immuno-compromised C3HeB/FeJ mice)**



Unpublished data from Gyanu Lamichhane's Lab at Johns Hopkins University.
PBS = Phosphate-buffered saline (negative control); IMI = Imipenem 100 mg/kg SC BID; EBO25 = Epetraborole 25 mg/kg PO QD; EBO50 = Epetraborole 50 mg/kg PO QD.

CFU = Colony-forming units; Mab = *Mycobacterium abscessus*; MIC = Minimum inhibitory concentration.

Epetraborole

PK & Tolerability Data Available Across Wide Dose Range (250–6,000 mg Daily)

- **Anacor/GSK: 6 "Legacy" Phase 1 studies**
  - 4 IV studies and 2 oral studies conducted in 2010-2011
  - High EBO doses studied, up to 6000 mg daily
- **AN2: 4 Phase 1 studies** (oral formulation)
  - **EBO-101:** Dose-ranging safety, PK & food effect of oral EBO 250–1000 mg daily up to 28 days; completed [2]
  - **EBO-102:** Renal impairment; completed
    - See **Poster #2144** (Sat, Oct 14, 12:15-1:30PM)
  - **EBO-103:** Ethnobridging study in Japan; completed
    - See **Poster #2556** (Sat, Oct 14, 12:15-1:30PM)
  - **EBO-104:** Thorough QT; enrollment completed
    - **ClinicalTrials.gov NCT05995444**
- **Oral EBO dosage for NTM lung disease is 500 mg QD**

Phase 1 Study	EBO Subjects (n)
IV Formulation	
SAD/MAD	SAD: 30 MAD: 24
Intrapulmonary PK [1]	Single dose: 15 q12h x 3 days: 15
SAD/MAD in Japan*	8
Mass balance	6
Total IV	**98**
Oral Formulation	
SAD/MAD	SAD: 19 MAD: 41
Food effect*	24
EBO-101: Dose-ranging x 28 days	39
EBO-102: Renal impairment	40
EBO-103: Ethnobridging	18
EBO-104: Thorough QT	24
Total Oral	**205**
TOTAL IV + Oral	**303**

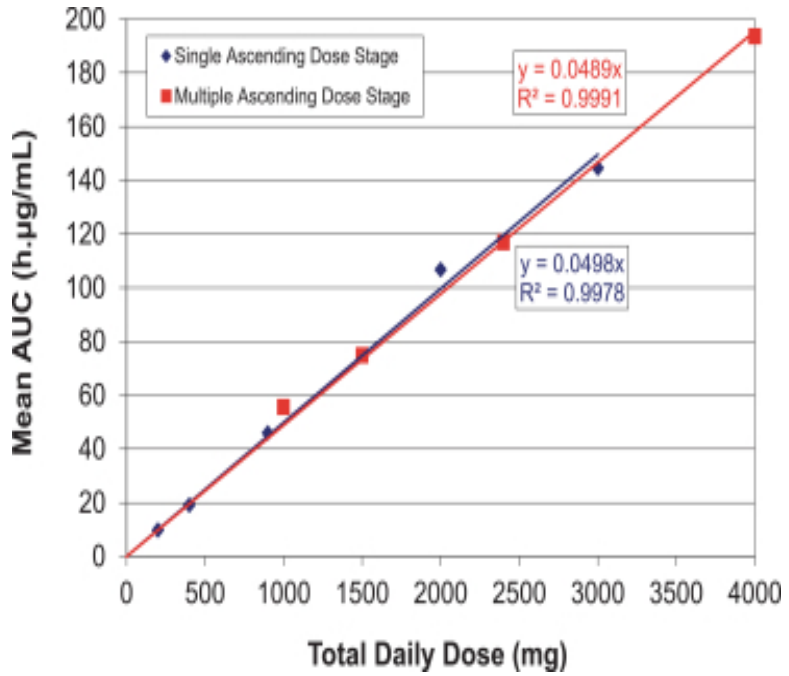
*Phase 1 studies terminated early due to discontinued Phase 2 cUTI program.

cUTI = Complicated urinary tract infection; EBO = Epetraborole; GI = Gastrointestinal; MAD = Multiple ascending dose; SAD = Single ascending dose.

[1] Tenero D, et al. *Antimicrob Agents Chemother* 2013;57:3334-3339. [2] Eckburg PB, et al. IDWeek 2022 Poster & Presentation.

Epetraborole
PK Characteristics

- **0% protein binding**
- **Metabolized to metabolite M3 by alcohol dehydrogenase (ADH)**
- **90% of dose (parent + M3) recovered in urine and ~8 % in feces**
- **Low systemic clearance (22–24 L/h),** indicating little first pass metabolism
- **Extensive tissue distribution;** volume of distribution 445 ±91 L for 500 mg QD
- **Linear kinetics observed following IV or oral doses of 250–4000 mg** (Figure)
- **T½ ~ 6–11 h**
- **Repeat dosing accumulation ratio for AUC is 0.99–1.24,** deemed not clinically meaningful
- **Mild food effect**
  - T_{max} increased, C_{max} decreased, and minor change in AUC



Correlation of mean EBO AUC to total daily IV EBO dose for SAD & MAD cohorts reveals a linear dose response.

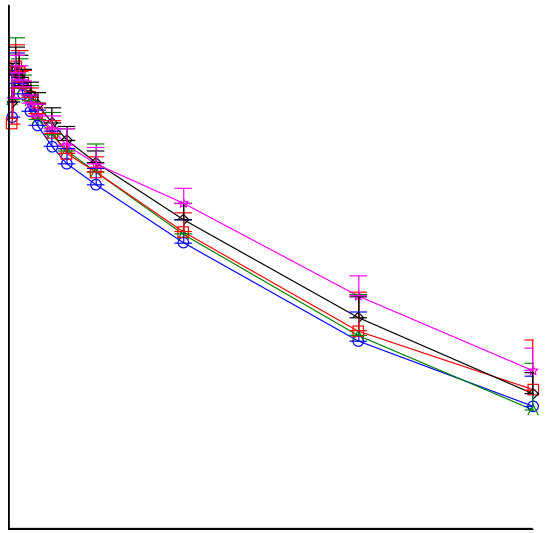
Zane LT, et al. 21st ECCMID 2011 Poster; Eckburg PB, et al. IDWeek 2022 Poster & Presentation.

EBO-102 | Phase 1 Renal Impairment Study

IDWeek 2023 Poster #2144

- **Objectives**: PK and safety of EBO in subjects with various degrees of renal impairment
- **Design**: Phase 1, multicenter, open-label, study; 5 dose cohorts, 8 subjects each, single-dose 500 mg QD
- **Cohorts:**

Cohort	Renal Impairment	N
1	Normal renal function (eGFR ≥ 90)	8
2	Mild (eGFR ≥ 60 and <90)	8
3	Moderate (eGFR ≥ 30 and <60)	8
4	Severe (eGFR <30)	8
5	ESRD on hemodialysis	8



Mean (+SD) plasma EBO concentration-time curves for Cohorts 1-5 in semi-log scale.

- **Results:**
  - EBO was well-tolerated
  - Subjects with renal impairment (Cohorts 2–5) did not exhibit quantitatively distinct EBO PK profiles
  - Increases in exposure of the inactive metabolite M3 were observed in subjects with severe renal impairment or ESRD on hemodialysis
  - Supports enrollment of patients with mild to moderate renal impairment in ongoing clinical trials

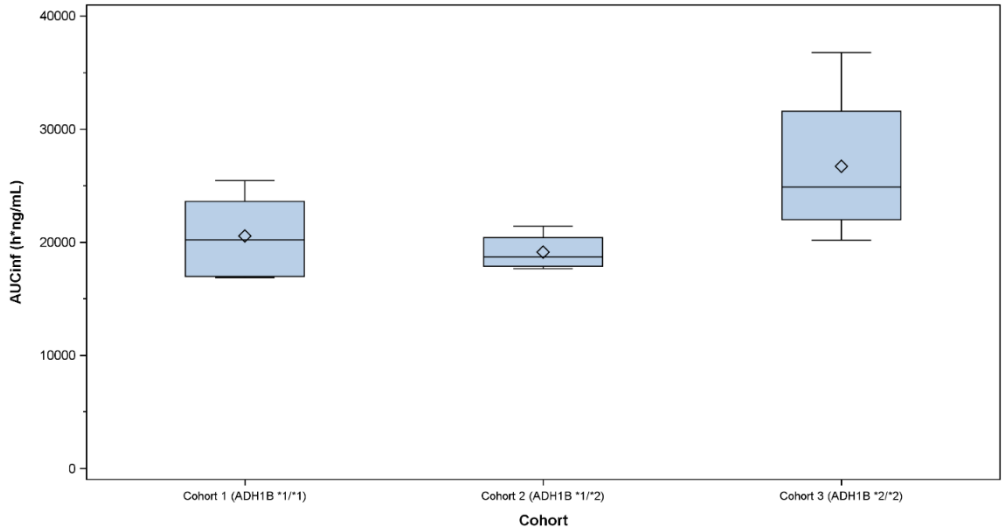
EBO = Epetraborole; eGFR = Estimated glomerular filtration rate in mL/min/1.73 m^2; ESRD = End-stage renal disease.

EBO-103 | Phase 1 Ethnobridging Study in Japan

IDWeek 2023 Poster #2556

- **Objectives**:
  - Assess the PK of EBO in Japanese subjects with various ADH1B genotypes
  - Assess EBO safety and tolerability
- **Design**: Phase 1, open-label, 3 dose cohorts, 6 subjects each, single-dose 500 mg
- **Cohorts:**

Cohort	ADH Genotype	Prevalence in Japan[1]	N
1	ADH1B *1/*1 (normal EtOH metabolism)	5%	6
2	ADH1B *1/*2 (moderately increased EtOH metabolism)	35%	6
3	ADH1B *2/*2 (fast EtOH metabolism)	60%	6



Box plots of EBO exposures ($AUC_{0\text{-}inf}$ in h*ng/mL) by cohort.

- **Results:**
  - EBO was well tolerated (no TEAEs)
  - EBO exposure in ADH1B*2/*2 subjects was ~1.2-1.4-folder higher than in ADH1B *1/*1 subjects
  - No underdosing of EBO predicted in patients with ADH1B *1/*2 or *2/*2 genotypes, including Japanese patients

ADH1B = Alcohol dehydrogenase type 1B; EBO = epetraborole; EtOH = Ethanol. [1]Eng MY, et al. *Alcohol Res Health* 2007;30:22-27.

Epetraborole

Well-tolerated Across 250–1000 mg QD x 28 Days

EBO-101 Phase 1 Dose-Ranging Study (QD x 28 days): Incidence of TEAEs occurring in ≥10% of subjects in oral EBO group (Safety Population).[1]

	Number (%) of Subjects/ [Number of Events]	
	Pooled Epetraborole (N=39)	**Pooled Placebo (N=12)**
	n (%)	**n (%)**
At least 1 TEAE	30 (76.9) [153]	10 (83.3) [50]
Drug-related TEAE	11 (28.2) [52]	5 (41.7) [13]
Serious TEAE	0	0
Severe TEAE	0	0
TEAE leading to treatment discontinuation	3 (7.7) [6]	0
TEAE leading to study withdrawal	1 (2.6) [1]	0
TEAEs Occurring in ≥10% of subjects*		
Nausea	9 (23.1) [9]	2 (16.7) [2]
Vascular access site pain	9 (23.1) [11]	3 (25.0) [3]
Headache	7 (17.9) [12]	3 (25.0) [3]
Vessel puncture site bruise	6 (15.4) [8]	2 (16.7) [5]
Back pain	5 (12.8) [5]	0
Decreased appetite	4 (10.3) [4]	0
Diarrhea	4 (10.3) [6]	1 (8.3) [1]
Upper respiratory tract infection	4 (10.3) [4]	1 (8.3) [1]

*One TEAE of anemia (predefined TEAE of special interest) occurred in a single EBO 1000 mg PO QD subject.

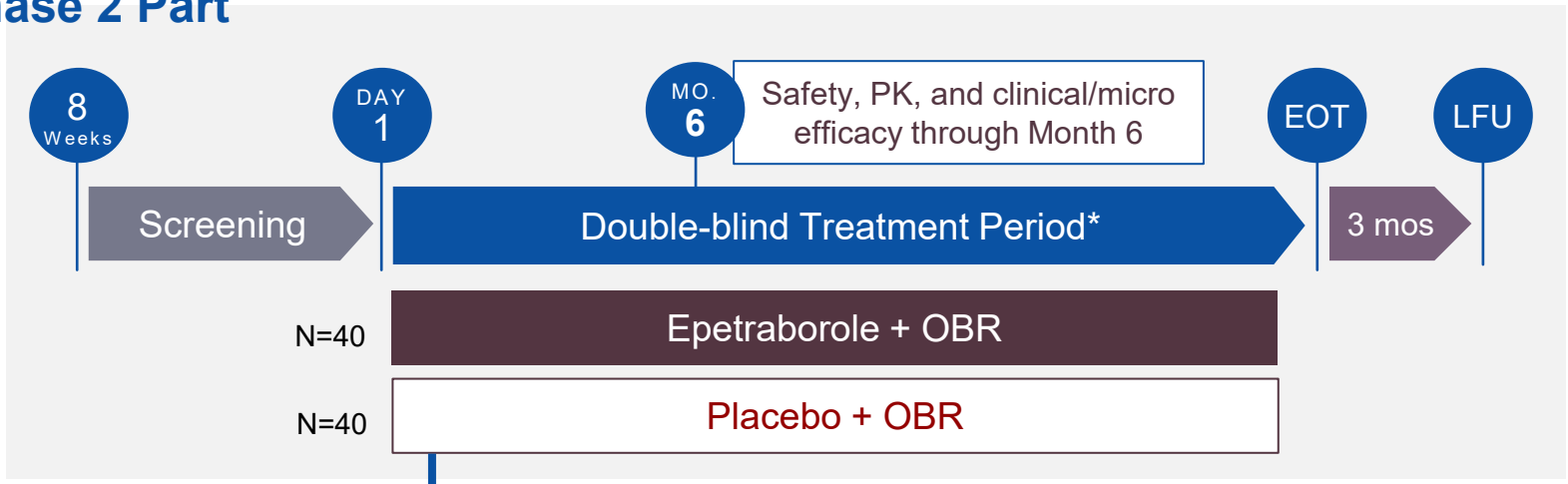
- **92% TEAEs mild, most commonly mild GI events**
  - 41.0% EBO vs. 41.7% placebo
  - No cases of *C. difficile*
- **EBO 500 mg QD (dosage under study in MAC lung disease) was well tolerated in healthy volunteers**
- **Current Phase 2 assessing safety beyond 28 days** (up to 16 months duration; next slide)

EBO = Epetraborole; GI = Gastrointestinal; QD = Once daily; TEAE = Treatment-emergent adverse event. [1] Eckburg PB, et al. IDWeek 2022 Poster.

EBO-301 ($MACrO_2$) | Phase 2/3 Trial in Treatment-refractory MAC Lung Disease

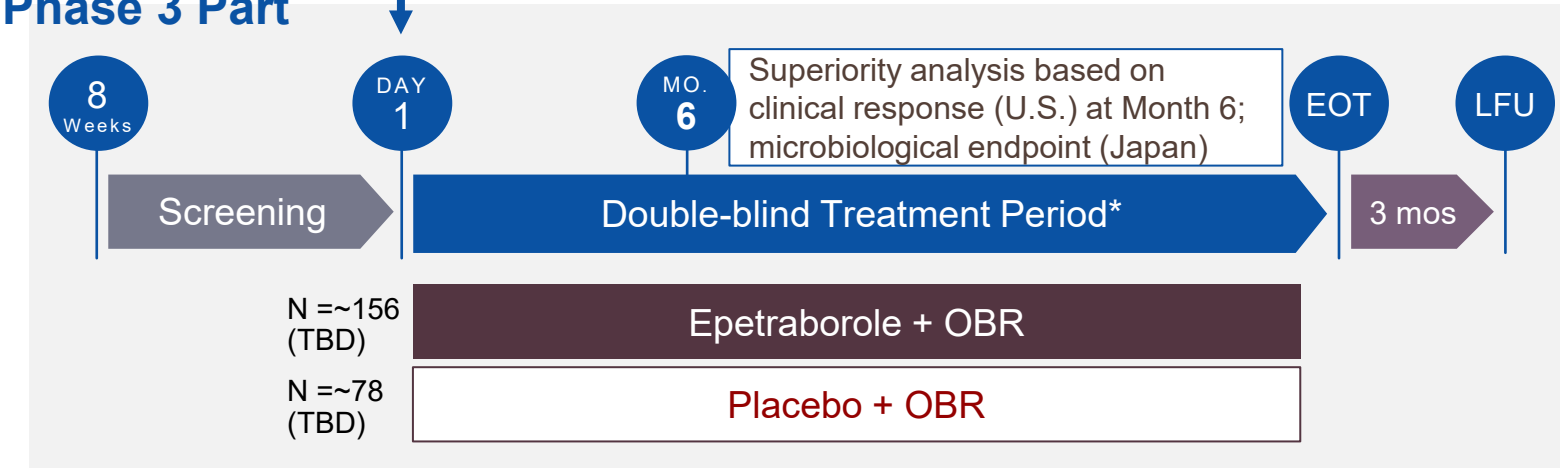
Phase 2 Enrolled; Currently Enrolling in Phase 3 Part

Phase 2 Part



Seamless enrollment

Phase 3 Part



For more information, see
macro2study.com &
ClinicalTrials.gov
NCT05327803

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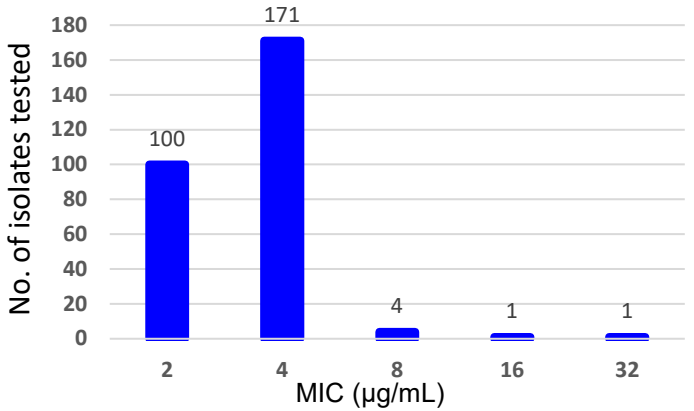
* Patients who culture convert will be treated for 12 months from 1st negative culture per treatment guidelines. EOT = End-of-Therapy; LFU = Late Follow-up; OBR = Optimized Background Regimen, consisting of at least 2 antimycobacterial agents; TBD = Final Phase 3 sample size to be determined based on Phase 2 data.

AN2's Global Health Commitment

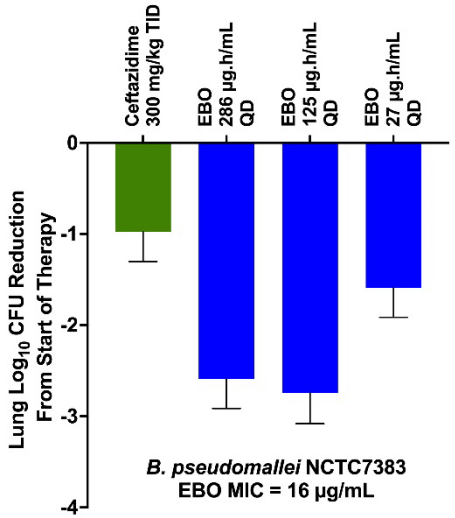
Epetraborole is a Promising Agent vs. Melioidosis

- **Developing IV EBO for intensive phase therapy**
  - ~165,000 cases and 89,000 deaths per year worldwide, with >50% all-cause mortality [1]
  - One of deadliest neglected tropical diseases, with global burden of 4.6 million disability-adjusted life years [2]
  - Endemic in Southeast Asia, India & Northern Australia
  - Initial focus on hospitalized patients with acute systemic disease, in combination with SOC (e.g., ceftazidime)
- **Phase 3-enabling nonclinical and clinical studies funded by NIAID** (up to $17.8M contract)
- **Partnering with world melioidosis experts at MORU**
  - **Multicenter, prospective, observational study** underway to characterize Thai & Lao patients with suspected melioidosis and assess potential clinical endpoints for Phase 3

EBO MICs for 277 clinical isolates of *B. pseudomallei* from 2019 (unpublished data from MORU)



Acute pulmonary infection model of melioidosis in BALB/c mice (unpublished data from CSU)



- Drugs were administered 24 hours after infection by subcutaneous injections over 48 hours
- EBO AUC_{0-24} for 1500 mg q12h IV in humans was 146 µg.h/mL[3]

CSU = Slayden Lab at Colorado State University; EBO = Epetraborole; MIC = Minimum inhibitory concentration; MORU = Mahidol-Oxford Research Unit.

[1] Limmathurotsakul D, et al. *Nat Microbiol* 2016;1:1. [2] Birnie E, et al. *Lancet Infect Dis* 2019;19:892-902. [3] Tenero D, et al. *AAC* 2013;57:3334-3339.

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Thank You!