Poster No. 1727
IDWeek 2022
Washington, D.C.
October 19–23, 2022

Tolerability and Pharmacokinetics of Oral Epetraborole at the Predicted Therapeutic Dosage for *Mycobacterium avium* Complex (MAC) Lung Disease: A Phase 1b Dose-ranging and Food Effect Study

Paul B. Eckburg, MD;[1] Dave Clarke, PhD;[1] Jennifer Long, MSPH;[1] Sanjay Chanda, PhD;[1] Kevin M. Krause, MBA;[1] Eric Easom, MBA;[1] George Talbot, MD;[2] Chris Rubino, PharmD;[3] Angela Molga, MD[4]

[1] AN2 Therapeutics, Menlo Park, CA [2] Talbot Advisors, LLC, Anna Maria, FL [3] Institute of Clinical Pharmacodynamics, Schenectady, NY [4] Department of Clinical Pharmacology, Royal Adelaide Hospital, Adelaide, Australia

AN2Therapeutics
Menlo Park, CA 94027
www.an2therapeutics.com

ABSTRACT

Background: Epetraborole (EBO) — an orally available bacterial leucyl transfer RNA synthetase inhibitor with potent activity against nontuberculous mycobacteria (NTM) — is under clinical development for treatment of *Mycobacterium avium* complex lung disease (MAC-LD). We conducted a Phase 1b dose-ranging study of EBO tablets in healthy adult volunteers, to inform dose selection in the treatment of MAC-LD lung disease.

Methods: In this double-blind, placebo-controlled trial, EBO or placebo tablets were administered (n=8/cohort, 3:1 randomization) at dosages of 250-1000 mg q24h or 500 mg or 1000 mg q48h for up to 28 days. Standard Phase 1 clinical and laboratory evaluations and treatment-emergent adverse events (TEAEs) were assessed. Based on prior human studies using significantly higher EBO daily doses, gastrointestinal (GI) events and anemia were predetermined AEs of special interest (AESIs). Plasma concentrations of EBO were measured by validated LC-MS/MS methods. Plasma PK parameters were determined using non-compartmental methods.

Results: A total of 43 subjects were enrolled; the 1000 mg q24h cohort was terminated early due to local COVID restrictions. Overall, 80.6% EBO subjects and 83.3% placebo subjects experienced ≥1 TEAE, none of which was serious or severe. Most TEAEs were mild in severity (92%), and the remainder were moderate (8%). The most frequent types of TEAEs were GI events (48.4% EBO, 41.7% placebo subjects), the most common being mild nausea. Two subjects had premature discontinuation of EBO due to a TEAE (asymptomatic liver enzyme elevations in a 250 mg q24h subject and mild nausea in a 1000 mg q48h subject). One 1000mg q24h subject had a TEAE of anemia. No clinically significant findings or TEAEs were observed for physical examinations, ECGs, or urine laboratory tests. Plasma C_{max} and $AUC_{0-\infty}$ of EBO increased in a linear, dose-proportional manner across cohorts. T_{max} was observed at ~1 h post dose; mean $t_{½}$ ranged from 7.63 to 11.1 h.

Conclusion:
- Oral EBO administered for 28-day dosing was generally well tolerated at the predicted therapeutic dose (500mg q24h)
- Predictable PK characteristics facilitate its use in MAC lung disease
- Further evaluation in a Phase 2/3 treatment-refractory MAC lung disease study is planned

INTRODUCTION

- Nontuberculous mycobacterial lung disease (NTM-LD), most commonly due to MAC, is a serious chronic, progressive infection caused by inhaled mycobacteria from environmental sources (Bethencourt and Ferrer, 2020).
- The prevalence of NTM-LD is increasing, and its occurrence is associated with higher healthcare costs and poor clinical outcomes (Bethencourt 2020; Abate 2020; Baldwin 2019; Horne 019; Marras 2018; Marras 2019; Prevots 2015; Winthrop 2020).
- Despite prolonged therapy with antibiotic combinations, treatment is often complicated by adverse effects that contribute to high relapse rates (Abate 2020; Jarand 2016; Deshpande 2011; Marras 2018a.
- An unmet need exists for more effective treatment options for NTM-LD with improved safety and tolerability profiles.
- EBO is being developed as a novel, oral therapy for treating NTM-LD.
- EBO blocks bacterial protein synthesis by inhibiting leucyl-tRNA synthetase.
- EBO is active *in vitro* vs. NTM, including isolates resistant to drugs commonly used to treat MAC (e.g., clarithromycin, amikacin), with minimum inhibitory concentration (MIC) values of 0.25 to 8 µg/m.

OBJECTIVE

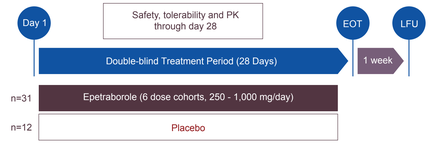
Evaluate the safety, tolerability, pharmacokinetics (PK), and food effect of EBO administered for up to 28 days in healthy adult subjects to provide information on dose selection for clinical studies in MAC-LD.

METHODS

Study Design

- This was a Phase 1b dose-ranging and food effect study (**Figure 1**)
  - Screening visit within 28 days of 1st dose
  - Double blind, randomized, placebo-controlled, dose-ranging part with 28-day dosing q24h or q48h. (Cohorts 1 to 6)
  - Single-dose (500 mg) open-label food effect Cohort (Cohort 7).
- All subjects were confined to a clinical research unit for the duration for the study.

Figure 1. Study Design of Dose-ranging Cohorts 1-6.



Subject Eligibility Criteria

- Healthy adult males or females of 18 to 65 years of age (inclusive)
- Body weight between 40.0 kg and 100.0 kg and body mass index (BMI) of ≥18.0 and ≤30.0 kg/m^2
- Medically healthy without clinically significant physical examination or laboratory abnormalities at Screening or Day -1

Study Assessments

- Safety was assessed from TEAEs, physical examination, vital signs (heart rate, blood pressure, respiratory rate), 12-lead electrocardiograms (ECGs), and clinical laboratory tests (hematology, coagulation, serum chemistry, and urinalysis)

Pharmacokinetic Analysis

- Plasma concentrations of EBO and metabolite M3 were measured with a validated HPLC-MS/MS assay
- PK parameters were determined for EBO and M3

Statistical Analysis

- Dose proportionality was determined for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$
- Food effect was assessed with a random effects mixed model on log-transformed AUC_{0-t}, $AUC0-\infty$, and C_{max}, where fed vs. fasted was a fixed effect and subject was a random effect
- Geometric means for fed/fasted and 90% CIs were estimated based on least square means
- An absence of food effect was not established if the 90% CI for the geometric mean ratio between fed and fasted conditions was not contained in the equivalence limits of 0.8 to 1.25 for AUC_{0-t}, AUC_{0-inf} or C_{max}

RESULTS

- A total of 51 subjects were enrolled across 7 dose cohorts
  - 39 subjects received EBO and 12 received placebo
- Cohort 6 (EBO 1000 mg q24h) was terminated after two subjects were enrolled, because of the local impact of COVID on continued study conduct.
- 48 (94.1%) subjects completed the study and were included in the safety population
  - 1 (2.6%) subject in Cohort 5 withdrew from study due to a family emergency
  - 1 (2.6%) in Cohort 7 withdrew from study due to an unrelated TEAE of mild tooth infection
  - 1 (2.6%) in the placebo cohort withdrew from study due to a family issue
- 37 subjects were in the PK population
- Baseline characteristics were similar among cohorts, except a higher percentage of placebo subjects were male (**Table 1**)

Table 1. Baseline characteristics

Parameter	Pooled EBO (N=39)	Pooled Placebo (N=12)
Age, years (mean ± standard deviation)	32.0 ± 10.9	36.0 ± 12.7
Age range, years	18 – 61	22 – 59
Male	27 (69.2)	11 (91.7)
Race n (%)		
Asian	6 (15.4)	3 (25.0)
White	32 (82.1)	8(66.7)
Other	2 (5.1)	1 (8.3)
Ethnicity n (%)		
Hispanic or Latino	4 (10.3)	1 (8.3)
Not Hispanic or Latino	34 (87.2)	11 (91.7)
Unknown	1 (2.6)	0

Pharmacokinetics

Dose-ranging cohorts (Dose Cohorts 1 to 6)

- Rate and extent of systemic exposure of EBO and metabolite M3 measured by C_{max} and AUC (AUC_{0-t} and AUC_{0-tau}) were dose linear (**Tables 2 and 3**)
- EBO and M3 reached C_{max} rapidly (**Figures 1 and 2**)
- Mean accumulation ratios for AUC_{0-24} and Cmax were generally consistent across dose

Table 2. EBO PK Parameters at Day 1 and Day 28 for Dose Cohorts 1 to 6 (PK Population)

Dose Cohort	EBO Dose Regimen	N	AUC_{0-12} (h*ng/mL)[a]	AUC_{0-24} (h*ng/mL)[a]	C_{max} (ng/mL)[a]	$t_{½}$ (h)[a]	T_{max} (h)[b]
DAY 1							
1	250 mg q24h	6	5630± 1010	6950±1160	1440 ± 161	8.64±1.06	1.03 (0.5 to 2.0)
2	500 mg q48h	6	12100±2290	14900±2890	3800 ± 1770	11.7±1.13	1.08 (1.0 to 1.5)
3	500 mg q24h	6	9840±1730	12300±2310	2620 ± 545	8.48±1.45	1.0 (0.5 to 2.0)
4	750 mg q24h	6	16400±4380	20000±5810	5600 ± 2130	8.49±1.99	1.0 (0.5 to 2.0)
5	1000 mg q48h	6	19100±2780	23400±3820	5300 ± 1300	10.6±1.78	1.0 (0.5 to 2.0)
6	1000 mg q24h	1	24700	29200	4930	5.91	1.00
7 fasted	500 mg q24h	8	12700 ± 2600	15200 ± 3190	3820 ± 1310	8.94 ± 0.961	1.0 (0.5 to 2.0)
7 fed	500 mg q24h	7	8910 ± 1060	11200 ± 1680	1750 ± 317	8.78 ± 1.41	2.0 (2.0 to 3.0)
DAY 28							
Dose Cohort	**EBO Dose Regimen**	**N**	**AUC_{0-12} (h*ng/mL)[a]**	**AUC_{0-24} (h*ng/mL)[a]**	**$C_{max,ss}$ (ng/mL)[a]**	**$t_{½}$ (h)[a]**	**$T_{max,ss}$ (h)[b]**
1	250 mg q24h	5	5730 ± 622	7100 ± 792	1390 ± 278	10.6 ± 0.966	1.5 (1.0 to 1.5)
2	500 mg q48h[c]	6	11600 ± 1500	14700 ± 1990	3150 ± 429	11.2 ± 1.12	1.0 (0.5 to 1.5)
3	500 mg q24h	6	11000 ± 1690	13700 ± 2380	2850 ± 275	10.2 ± 1.12	1.0 (0.5 to 1.5)
4	750 mg q24h	6	19400 ± 4370	24400 ± 6240	5280 ± 1430	10.3 ± 1.47	1.0 (0.5 to 1.5)
5	1000 mg q48h[c]	5	19600 ± 2290	24600 ± 3640	4810 ± 1220	10.8 ± 1.00	1.0 (1.0 to 1.5)
6	1000 mg q24h	1	26800	32200	5210	7.63	3.0

AUC_{0-12} = the area under the plasma drug concentration-time curve, from time 0 to 12 hours; AUC_{0-24} = the area under the plasma drug concentration-time curve, from time 0 to 24 hours C_{max} = observed maximum plasma drug concentration; $t_{½}$ = apparent plasma half life; T_{max} = time to reach maximum plasma drug concentration; $C_{max,ss}$ = observed maximum plasma drug concentration in the dosing interval at steady state; h = hour; N = number of subjects in Cohort; $T_{max,ss}$= time to reach maximum plasma drug concentration in the dosing interval at steady state.
[a] Data are presented as the arithmetic mean ± standard deviation.
[b] Data are presented as the median (range).
[c] Parameters are shown at Day 27 for Dose Cohorts 2 and 5 due to q48h dosing regimen.

Table 3. M3 PK Parameters at Day 1 and Day 28 for Dose Cohorts 1 to 6 (PK Population)

Dose Cohort	EBO Dose Regimen	N	AUC_{0-12} (h*ng/mL)[a]	AUC_{0-24} (h*ng/mL)[a]	C_{max} (ng/mL)[a]	$t_{½}$ (h)[a]	T_{max} (h)[b]
Day 1							
1	250 mg q24h	6	15500 ± 823	21500 ± 859	2230 ± 203	11.6 ± 3.10	3.0 (2.0 to 4.0)
2	500 mg q48h	6	23000 ± 3860	34200 ± 5340	3050 ±741	19.9 ± 1.34	2.5 (1.5 to 3.2)
3	500 mg q24h	6	24500 ±3340	35100 ± 3520	3630 ± 732	15.3 ± 4.24	2.0 (2.0 to 3.0)
4	750 mg q24h	6	25100 ± 3970	38500 ± 6650	3270 ± 457	23.9 ± 15.8	2.0 (1.5 to 3.0)
5	1000 mg q48h	6	29400 ± 2760	46100 ± 4400	3640 ± 592	23.0 ± 1.97	2.5 (1.5 to 3.0)
6	1000 mg q24h	1	30200	47400	4080	19.6	3.0
7 fasted	500 mg	8	22000 ± 3380	32700 ± 5070	3110 ± 580	18.7 ± 2.63	2.1 (2.0 to 3.0)
7 fed	500 mg	8	16200 ± 1440	25100 ± 2850	2220 ± 248	19.0 ± 3.23	4.0 (4.0 to 6.2)
Day 28			**AUC_{0-12} (h*ng/mL)[a]**	**AUC_{0-24} (h*ng/mL)[a]**	**$C_{max,ss}$ (ng/mL)[a]**	**$t^{1}/_{2}$ (h)[a]**	**$T_{max,ss}$ (h)[b]**
1	250 mg q24h	6	20500 ± 10200	30100 ± 14800	2820 ±1470	34.5 ± 24.2	2.0 (2.0 to 3.0)
2	500 mg q48h[c]	6	29800 ± 4930	45600 ± 6960	3870 ± 595	22.9 ± 2.69	2.0 (1.50to 2.0)
3	500 mg q24h	6	38400 ± 5800	58700 ± 9080	5030 ± 775	25.5 ± 2.98	2.0 (1.5 to 3.0)
4	750 mg q24h	6	49400 ± 6420	79600 ± 10500	5600 ± 830	22.6 ± 1.18	2.0 (1.5 to 3.0)
5	1000 mg q48h[c]	5	40900 ± 4360	66800 ± 8720	4640 ± 535	26.6 ± 4.28	3.0 (1.5 to 4.0)
6	1000 mg q24h	1	63500	101000	8010	23.7	3.0

[a] Data are presented as the arithmetic mean ± standard deviation.
[b] Data are presented as the median (range).
[c] Parameters are shown at Day 27 for Dose Cohorts 2 and 5 due to q48h dosing regimen.

Figure 1. Mean EBO Plasma Concentration (semi-log) for Dose Cohorts 1 to 6 (PK Population) [CSR Figure 2]

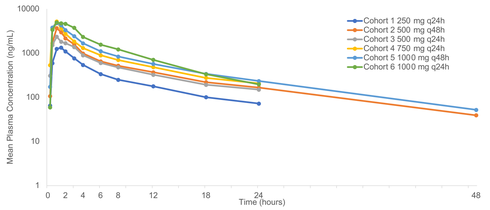
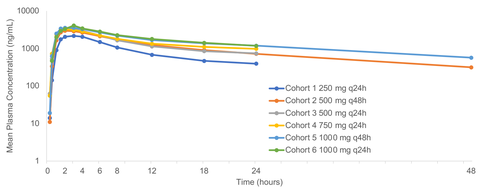


Figure 2. Mean M3 Plasma Concentrations (Semi-logarithmic) for Dose Cohorts 1 to 6 (PK Population)



Food effect cohort (Dose Cohort 7)

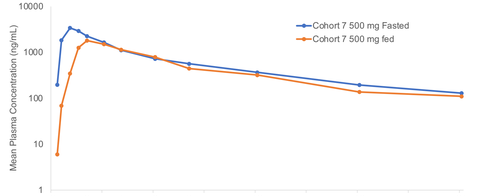
- Systemic absorption of EBO and metabolite M3 after a 500 mg dose was rapid regardless of fed or fasting conditions (**Figure 3**)
- The $t_{½}$ of EBO was comparable under fed and fasting conditions (**Table 4**)
- An absence of food effect on bioavailability was not established for EBO and M3 since the 90% CI for the geometric mean ratio between fed and fasted conditions was not contained in the equivalence limits of 0.8 to 1.25 for AUC_{0-t}, $AUC_{0-\infty}$, or C_{max}

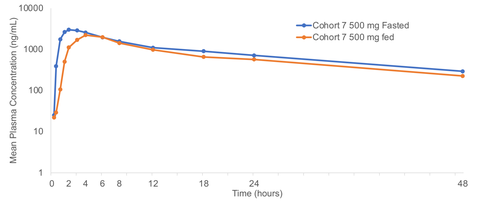
Table 4. EBO and metabolite M3 plasma PK parameters under fasting and fed conditions after a 500 mg dose of EBO (Cohort 7, PK population)

Dose Cohort	N	AUC_{0-12} (h*ng/mL)[a]	AUC_{0-24} (h*ng/mL)[a]	C_{max} (ng/mL)[a]	$t_{½}$ (h)[a]	T_{max} (h)[b]
EBO						
Fasted	8	12700 ± 2600	15200 ± 3190	3820 ± 1310	8.94 ± 0.961	1.0 (0.5 to 2.0)
Fed	7	8910 ± 1060	11200 ± 1680	1750 ± 317	8.78 ± 1.41	2.0 (2.0 to 3.0)
Metabolite M3						
Fasted	8	22000 ± 3380	32700 ± 5070	3110 ± 580	18.7 ± 2.63	2.1 (2.0 to 3.0)
Fed	8	16200 ± 1440	25100 ± 2850	2220 ± 248	19.0 ± 3.23	4.0 (4.0 to 6.2)

Abbreviations: AUC_{0-12} = the area under the plasma drug concentration-time curve, from time 0 to 12 hours; AUC_{0-24} = the area under the plasma drug concentration-time curve, from time 0 to 24 hours; C_{max} = observed maximum plasma drug concentration; h = hour; N = number of subjects in Cohort; q24h = every 24 hours; $t_{½}$ = apparent plasma half life; T_{max} = time to reach maximum plasma drug concentration.
[a] Data are presented as the arithmetic mean ± standard deviation.
[b] Data are presented as the median (range).

Figure 3. Mean EBO and Metabolite M3 Plasma Concentration (semi-log) During Fed and Fasted States (PK Population)





Safety/Tolerability

- EBO was generally well tolerated (**Table 5**)
- TEAEs were similar for pooled EBO and placebo groups
- Most TEAEs were mild in severity (92%) and the remainder were moderate (8%); there were no severe TEAEs
- No serious TEAEs were observed, including life-threatening TEAE or death
- Gastrointestinal (GI) disorders were the most common TEAEs (41.0% EBO subjects, 41.7% placebo subjects):
  - Nausea was the most common GI TEAE, most cases of which were mild
  - Diarrhea was less common, generally described as mild loose stools
  - No case of *Clostridioides difficile* infection
  - EBO 500 mg q24h — the dosage selected for future MAC-LD studies — was very well tolerated; only 1 subject experienced drug-related TEAEs (mild, non-treatment-limiting nausea and abdominal distension)
- No hemoglobin decrease led to discontinuation or interruption of study drug administration
  - 1 TEAE of moderate anemia occurred in the single EBO subject in the highest dose cohort (1000 mg q24h); no other TEAEs of anemia occurred at any other dose level
  - 5 other subjects experienced decreases of hemoglobin below the LLN of the normal reference range deemed NCS by the PI; however, some of these cases may have been confounded by concomitant TEAEs, such as upper respiratory tract infection, parainfluenza virus infection, iron deficiency, menstruation, and epistaxis
  - The general clinical signature of observed RBC abnormalities featured early decreases in reticulocyte levels, followed by gradual decreases in hemoglobin levels, with nadirs around Day 8 and Day 28, respectively. All decreases in RBC parameters were asymptomatic, normocytic and normochromic, and reversed rapidly after the last dose of study drug
- No clinically relevant changes in clinical laboratory (serum chemistry, coagulation, or urinalysis) results, vital signs, ECG or physical examination were observed

Table 5. Incidence of adverse events occurring in at least 10% of subjects in the oral EBO group (safety population)

	Number (%) of Subjects [Number of Events]	
	Pooled EBO (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[a]	3 (7.7)[6]	0
TEAE leading to study withdrawal[b]	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]

[a] One EBO 250 mg q24h subject with moderate AST, moderate ALT increased, mild GGT increased, and mild blood alkaline phosphatase increased; 1 EBO 1000 mg q48h subject with mild nausea; and 1 EBO 500 mg fasting subject with Mild unrelated tooth infection
[b] Mild unrelated tooth infection

SUMMARY AND CONCLUSIONS

- EBO is a novel oral antibiotic with a unique mechanism of action. It is being developed for the treatment of serious bacterial infections, including NTM-LD.
- Following oral administration of EBO 250 to 1000 mg, the rate and extent of systemic exposure of EBO and metabolite M3 were dose linear.
- Absorption of EBO was rapid, and PK parameters of $t^{1}/_{2}$, plasma clearance, and volume of distribution were similar between fasting and fed conditions.
- An absence of food effect on EBO bioavailability was not established for EBO and metabolite M3.
- Oral EBO administered for 28-day dosing was generally well tolerated at the predicted therapeutic dose (500mg q24h). The majority of TEAEs were GI events, most commonly mild nausea.
- No new safety concerns and no new risk were observed that would preclude evaluation of EBO in the target population of patients.
- These data support future clinical studies of EBO in the treatment of MAC-LD.

REFERENCES

- Abate G, et al. Variability in the management of adults with pulmonary nontuberculous mycobacterial disease. Clin Infect Dis. 2020. pii: ciaa252.
- Baldwin SL, et al. The complexities and challenges of preventing and treating nontuberculous mycobacterial diseases. PLoS Negl Trop Dis 2019;13(2): e0007083.
- Bethencourt Mirabal A, Ferrer G. Lung nontuberculous mycobacterial infections. 2019 Nov 16. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan.
- Deshpande D, Gumbo T. Pharmacokinetic/pharmacodynamic-based treatment of disseminated Mycobacterium avium. Future Microbiol. 2011;6(4):433-439.
- Horne D, Skerrett S. Recent advances in nontuberculous mycobacterial lung infections. F1000Res. 2019;8. pii: F1000 Faculty Rev-1710.
- Jarand J, et al. Long-term follow-up of Mycobacterium avium complex lung disease in patients treated with regimens including clofazimine and/or rifampin. Chest. 2016;149(5):1285-1293.
- Marras TK, et al. Health care utilization and expenditures following diagnosis of nontuberculous mycobacterial lung disease in the United States. JMCP. 2018;24:964–974.
- Marras TK, et al. Guidelines-based treatment associated with improved economic outcomes in nontuberculous mycobacterial lung disease. J Med Econ. 2019;22(11):1126-1133.
- Marras TK, et al. Relative risk of all-cause mortality in patients with nontuberculous mycobacterial lung disease in a US managed care population. Respir Med. 2018a;145:80-88.
- Prevots DR, Marras TK. Epidemiology of human pulmonary infection with nontuberculous mycobacteria: a review. Clin Chest Med. 2015;36:13–34.
- Winthrop KL, et al. Incidence and prevalence of nontuberculous mycobacterial lung disease in a large U.S. managed care health plan, 2008-2015. Ann Am Thorac Soc. 2020;17(2):178-85.