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The microbiological and clinical complexity of infections in patients with treatment-refractory *Mycobacterium avium* complex lung disease

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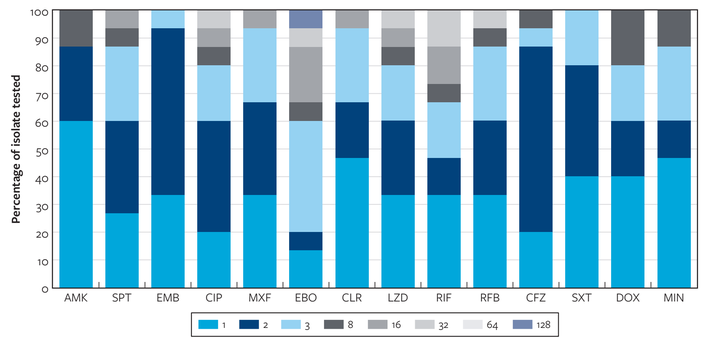
Introduction

- *Mycobacterium avium* complex (MAC) is comprised of two main species: *M. avium* and *M. intracellulare*.
- The incidence and prevalence of MAC lung disease have been increasing worldwide since 2000, with recent reports of 3.2-9.8 per 100,000 in North America and Australia (1).
- Current treatment guidelines for MAC consist of a macrolide (clarithromycin or azithromycin) with ethambutol and a rifamycin (2).
- Epetraborole (EBO) is a boron-containing inhibitor of bacterial leucyl-tRNA synthetase, an essential enzyme for protein synthesis.
- AN2 Therapuetics, Inc. recently concluded a Phase 2 clinical trial evaluating the effects of EBO in addition to an optimized background regimen (OBR) compared to placebo plus OBR against treatment-refractory patients with microbiological assessments of subject MAC isolates.

Methods

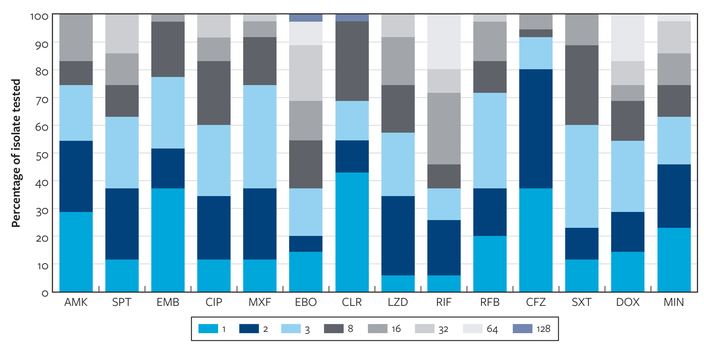
- A total of 80 adult patients with treatment-refractory MAC lung disease were enrolled and randomized 1:1 epetraborole + OBR vs. placebo + OBR.
- Multiple samples (1-3) were collected from patients at 8 time points and initially processed by a regional laboratory prior to shipment to Element Iowa City for testing.
- Isolate samples that displayed multiple morphologies were purified and banked as separate isolates from the same sample.
- All isolates were identified by MALDI-TOF MS and/or DNA sequencing.
- Isolates were tested by CLSI reference broth microdilution method (M24).
- CLSI breakpoints (M24S) were applied for comparator agents, where available.
- Isolates from samples containing multiple morphologies were considered identical if all MICs were -1±$\log_2$ dilution across all study antibiotics.

Figure 1. MIC shifts observed with multiple morphologies of *M. avium* recovered at screening



Abbreviations: AMK, amikacin; SPT, streptomycin; EMB, ethambutol; CIP, ciprofloxacin; MXF, moxifloxacin; EBO, epetraborole; CLR, clarithromycin; LZD, linezolid; RIF, rifampin; RFB, rifabutin; CFZ, clofazimine; SXT, trimethoprim/sulfamethoxazole; DOX, doxycycline; MIN, minocycline

Figure 2. MIC shifts observed with multiple morphologies of *M. intracellulare* recovered at screening



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Results

- Viable MAC isolates were recovered from a total of 79 patients, resulting in 1,030 primary isolates from all collected time points.
  - Primary isolates were believed to be single purified MAC colonies from solid agar plating.
- 303 primary isolates (29.4%) had ≥2 distinct morphologies (i.e., polyclonal) when plated onto Middlebrook 7H10 agar and susceptibility tested.
  - Multiple morphologies were identified in 62/79 patients (78.5%).
  - A total of 1,333 distinct isolates were identified.
  - 72.3% of samples with multiple morphologies were *M. intracellulare*.
    - 52.5% of *M. intracellulare* primary isolates had ≥2 distinct morphologies.
  - Baseline samples yielded 1-3 distinct isolates
- The *in vitro* activity of the study antibiotics against all distinct isolates is shown in Table 1.
  - *M. avium* isolates were observed to be 30–57% resistant to antibiotics, where breakpoints exist.
  - *M. intracellulare* isolates were observed to be 12–48% resistant to antibiotics, where breakpoints exist.
- The variability in the antimicrobial susceptibility patterns for multiple morphologies recovered from the same sample is shown for *M. avium* and *M. intracellulare* isolates as an MIC shift in Figure 1 and Figure 2, respectively.
  - The observed shifts for *M. intracellulare* were greater than those of *M. avium* across all of the tested antimicrobials.

Conclusions

- All patients enrolled in this study had a diagnosis of treatment-refractory MAC lung disease.
- 29.4% of the samples recovered from patients appear to contain polyclonal MAC infections, with isolates showing distinct morphologies and antibacterial susceptibility profiles.
- The microbiological complexity of the sputum samples and the range of MAC isolates identified for each patient and across the enrolled population complicated many of the study outcomes, including the determination of the primary pathogen for treatment-refractory MAC lung disease.
- EBO demonstrated promising activity against treatment-refractory MAC isolates with $MIC_{50/90}$ values of 2/16 mg/L against *M. avium* and 4/16 mg/L against *M. intracellulare* and are within the anticipated drug exposure targets for this trial.

Acknowledgments

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References

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Table 1. *In vitro* activity (mg/L) of study compounds against all distinct *M. avium* and *M. intracellulare* phenotypes recovered

		M. avium (n=679)						*M. intracellulare* (n=654)					
Antimicrobial	Class	MIC modal	MIC_{50}	MIC_{90}	%S	%I	%R	MIC modal	MIC_{50}	MIC_{90}	%S	%I	%R
AMK[a]	Aminoglycoside	>128	64	>128	64		36	16	32	128	88		12
AMK[b]	Aminoglycoside	>128	64	>128	20	26	54	16	32	128	46	21	33
SPT	Aminoglycoside	64	64	>64	N/A	N/A	N/A	>64	32	>64	N/A	N/A	N/A
EMB	Ethylenediamine	>32	32	>32	N/A	N/A	N/A	>32	16	>32	N/A	N/A	N/A
CIP	Fluoroquinolone	2	4	>16	N/A	N/A	N/A	>16	8	>16	N/A	N/A	N/A
MXF	Fluoroquinolone	2	2	>4	46	24	30	4	2	4	37	26	37
EBO	3-Aminomethyl benzoxaborole	0.5	2	16	N/A	N/A	N/A	8	4	16	N/A	N/A	N/A
CLR	Macrolide	>32	4	>32	63	3	34	>32	2	>32	73	3	24
LZD	Oxazolidinone	>16	>16	>16	20	23	57	>16	16	>16	33	19	48
RIF	Rifamycin	>8	>8	>8	N/A	N/A	N/A	>8	8	>8	N/A	N/A	N/A
RFB	Rifamycin	≤0.06	0.12	1	N/A	N/A	N/A	≤0.06	0.12	0.5	N/A	N/A	N/A
CFZ	Riminophenazine	≤0.25	0.5	1	N/A	N/A	N/A	≤0.25	≤0.25	1	N/A	N/A	N/A
SXT	Sulfonamide	≤0.5	≤0.5	4	N/A	N/A	N/A	≤0.5	1	>4	N/A	N/A	N/A
DOX	Tetracycline	>16	>16	>16	N/A	N/A	N/A	>16	>16	>16	N/A	N/A	N/A
MIN	Tetracycline	>8	8	>8	N/A	N/A	N/A	>8	>8	>8	N/A	N/A	N/A

[a] Liposomal, inhaled breakpoints applied
[b] IV breakpoints applied
MIC values in mg/L
Results are inclusive of all isolates collected from each timepoint, including samples with multiple morphologies
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