Poster #323

MICROBION CORPORATION

BisEDT, A Novel Small Molecule Drug, Demonstrates Broad Antimicrobial And Anti-Biofilm Activity Against CF Lung Pathogens

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ABSTRACT

Chronic polymicrobial bacterial lung infections in CF patients are major contributors to CF lung disease morbidity and mortality. Repeated courses of inhaled and intravenous antibiotics are required to control these infections and to preserve lung function, but this cumulative antibiotic exposure increases the risk of developing antibiotic resistance. Furthermore, current therapeutics have limited activity against chronic pulmonary bacterial biofilms. Antibacterial agents with novel mechanisms of action and activity against bacterial biofilms are needed.

BisEDT is a member of a novel class of bismane antimicrobials with broad activity against both Gram-positive and Gram-negative bacterial pathogens and strong antibiofilm properties. In several studies supported by the CF Foundation (CFF), we investigated BisEDT's activity against clinically relevant CF pathogens. Using a broth microdilution assay for minimum inhibitory concentrations (MICs), we found that BisEDT had broad antimicrobial activity against CF patient bacterial isolates representing MDR *Pseudomonas aeruginosa* (MIC≤1 mcg/mL), *Stenotrophomonas maltophilia* (0.25 mcg/mL), *Burkholderia* sp.(≤8 mcg/mL), *Achromobacter* sp. (≤1 mcg/mL), *Mycobacterium abscessus* complex (≤0.5 mcg/mL) and *Mycobacterium avium* complex (≤8 mcg/mL), including macrolide resistant Mycobacterial strains. We also performed bactericidal kill assays against *P. aeruginosa* in the presence of CF patient sputum, and observed that BisEDT retained bactericidal activity at the MIC. Biofilm eradication assays, demonstrated ≥99.9% bactericidal/antibiofilm activity against *Achromobacter*, multidrug resistant *P. aeruginosa*, *Burkholderia*, and *M. abscessus* grown as biofilms on plastic peg supports. Additionally, BisEDT was non-toxic in a cultured human airway epithelium tissue toxicology model at concentrations many fold higher than the MIC.

In subsequent non-CFF supported activities, Microbion formulated BisEDT as a clinically and commercially viable product to provide rapid delivery of efficacious doses to both the central and peripheral lung. After inhalation delivery to rats, BisEDT has a long lung residence time, which is anticipated to provide long residual activity and provide favorable dosing schedule flexibility. Inhalation delivery of well-tolerated doses of BisEDT in a rat *P. aeruginosa* agar bead lung infection model demonstrated statistically significant efficacy by reducing lung tissue CFU/g similarly to the high-dose positive control. In addition, BisEDT has demonstrated an excellent safety profile when delivered topically or intra-surgically to over 325 clinical study subjects with other clinical indications (healthy volunteers, patients with diabetic foot ulcer infections or orthopedic infections).

BisEDT offers strong potential as an inhaled antimicrobial/antibiofilm agent for suppression and treatment of multiple pathogens contributing to polymicrobial lung infections, including MDR *P. aeruginosa* and *M. abscessus* complex.

ABOUT PRAVIBISMANE (BisEDT)

- Broad-spectrum antimicrobial, antibiofilm agent with activity against all important CF-associated lung infection pathogens and a unique mechanism of action (MOA)
- Formulated for nebulized delivery for treatment of lung infections
- No known instances of cross-resistance and very low propensity for development of resistance
- WHO recommended INN for BisEDT is “pravibismane”
- Pravibimane is the first member of a novel microbial bioenergetic inhibitor class
- Granted Qualified Infectious Disease Product (QIDP) and Fast-Track designation by FDA for 3 clinical indications including management of CF-related pulmonary infections

ACTIVITY AGAINST CF-PATIENT ISOLATES

BisEDT (pravibismane) demonstrated very low minimal inhibitory concentration (MIC) values against MDR *P. aeruginosa*, *Burkholderia* spp., *S. maltophilia*, and *Achromobacter* spp. [Table 1].

Against *M. abscessus*, BisEDT was more potent than amikacin and clarithromycin [Table 2].

Table 1: MIC values for BisEDT and comparator agents

Strains (# of strains tested)	MIC (µg/ml)	
	BisEDT	Comparator Drugs
P. aeruginosa (3)	1	AMK 16, TOB 32, CAZ 1, PIP 1, MEM ≤1, CIP 1
	0.5 1	AMK >64, TOB 128, CAZ >64, PIP 256, MEM 8, CIP 4 AMK >64, TOB 32, CAZ >64, PIP>256, MEM 16, CIP 4
B. cepacia complex (2)	0.25 0.5	AMK 64, CAZ 2, MEM 4, SXT<1 not tested
B. cepacia (1)	8	AMK>64, TOB 128, CAZ 4, MEM 4, SXT<1
B. cenocepacia (2)	2 0.5	AMK>64, TOB 128, CAZ 16, MEM 16, SXT>16 AMK>64
B. multivorans (1)	0.25	AMK>64, TOB 64, CAZ 4, MEM≤0.5, SXT 1
B. dolosa (1)	0.125	CAZ>64, MEM 32, SXT 8
S. maltophilia (1)	0.25	AMK 64
Achromobacter spp (2)	0.25 1	AMK 64 AMK>64, TOB 256, CAZ 64, MEM>32, CIP≤0.25, SXT≤1
Reference Strains		
S. aureus ATCC 29213	0.25 – 0.5	n/a
E. coli ATCC 25922	1	n/a
P. aeruginosa ATCC 27853	1 – 2	n/a

Comparator agents: AMK = amikacin, TOB = tobramycin, CAZ = ceftazidime, PIP = piperacillin, MEM = meropenem, CIP = ciprofloxacin, SXT = trimethoprim-sulfamethoxazole
MICs were determined by broth microdilution test methods described in CLSI M07-A9.

Table 2: MIC values for BisEDT and comparators against NTM clinical isolates

Strains (# of strains tested)	Resistance	MIC (µg/ml)		
		BisEDT	Amikacin	Clarithromycin
M. avium (3)		8	n.t.	4-32
M. abscessus/massiliense complex (1)	Macrolide^{-S}, AMK^{-S}	0.06	16	1
M. abscessus/massiliense complex (3)	Macrolide^{-S}, AMK^{-I}	0.06 – 0.25	32	1-2
M. abscessus/massiliense complex (1)	Macrolide^{-S}, AMK^{-R}	0.5	>64	2
M. abscessus/massiliense complex (1)	Macrolide^{-R}, AMK^{-I}	0.125	32	>32
M. abscessus/massiliense complex (3)	Macrolide^{-R}, AMK^{-R}	0.125-0.25	>64	>32
Reference strain ATCC19977 *M. abscessus* subsp. *abscessus*	Macrolide^{-R}, AMK^{-S}	0.06	8	>32

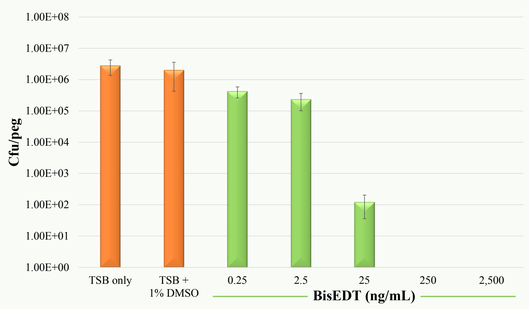
AMK = amikacin; S = susceptible; R = resistant; I = intermediate; n.t. = not tested. MICs are determined by broth microdilution test methods described in CLSI M24-A2, with addition of resazurin sodium for endpoint determination. For *M. abscessus/massiliense* complex clarithromycin MICs were determined after prolonged incubation for 14 days.

ANTI-BIOFILM ACTIVITY AGAINST CF-ISOLATES

- The MBEC Assay® was used to determine the efficacy of BisEDT against biofilms of CF-isolates
- Planktonic bacteria, grown under shear force, adhere to the surface of plastic pegs and form mature biofilms within 24-72 hrs
- Mature biofilms are exposed to serial dilutions of BisEDT for 24 hrs
- Following exposure, biofilms were dissociated from the pegs by sonication, then serially diluted, plated on LB agar plates, and grown for 24-72 hrs for quantitation of CFU

Biofilm eradication assays (MBEC) demonstrated ≥99.9% bactericidal/antibiofilm BisEDT (pravibismane) activity against multidrug resistant *P. aeruginosa*, *Burkholderia*, *Achromobacter* spp., and *M. abscessus* biofilms [Figs. 2 – 6].

Figure 2: MDR-*P. aeruginosa*; 48hr biofilms-24hr BisEDT treatment



TSB = tryptic soy broth

Figure 3: Aminoglycoside-resistant *P. aeruginosa*; 24hr biofilms-24hr BisEDT treatment

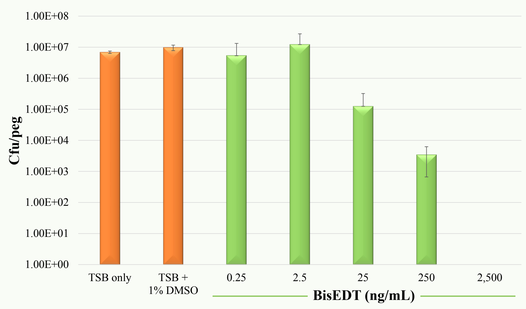


Figure 4: *Burkholderia cenocepacia*; 48hr biofilms-24hr BisEDT treatment

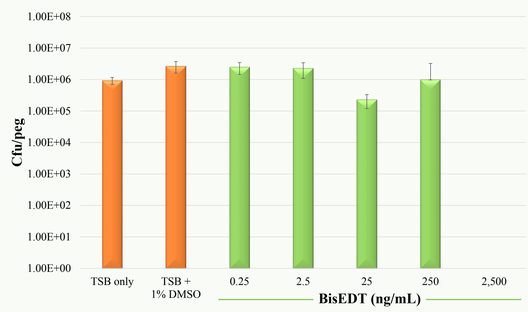


Figure 5: *Achromobacter* spp.; 24hr biofilms-24hr BisEDT treatment

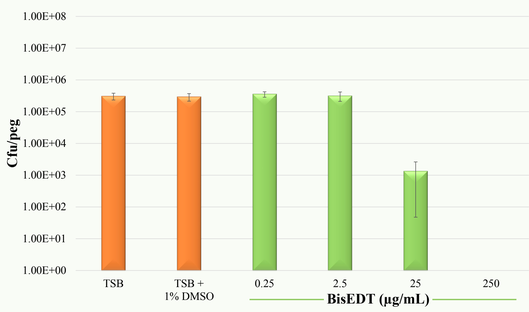
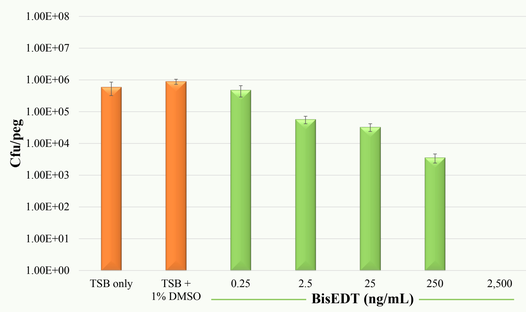


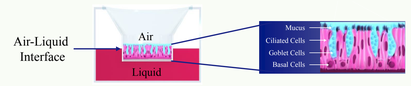
Figure 6: *M. abscessus* complex (CF isolate); 72hr biofilms-24hr BisEDT treatment



EFFECT ON CYTOTOXICITY ON HUMAN AIRWAY EPITHELIUM

- Toxicity to respiratory epithelium assessed with MucilAir™ [Fig. 7]
- De-differentiated epithelial cells from fresh nose and bronchi biopsies are seeded onto a semi-porous membrane
- After 45 days in culture, epithelia are ciliated, secrete mucus, and are electrically tight (TEER >200 Ω.cm^2)

Figure 7: MucilAir™ in vitro model of the human airway



- BisEDT was applied on the air (luminal) side as:
  - Solution [Figs. 8A and 8B]
  - Microtablet [Figs. 9A and 9B]
- Toxicity was monitored by:
  - LDH (lactate dehydrogenase) release [Figs. 8A and 9A]
  - TEER (impedance) [Figs. 8B and 9B]
  - Microscopic morphology (not shown)

Results:

- 0.33 µg/cm^2 (equivalent to 1,100 µg/mL*) BisEDT was not toxic to human airway epithelia
- No morphologic changes were observed with 0.33 µg/cm^2 BisEDT

* assumes a standard 3 µm thick surface fluid layer in human airway epithelium

BisEDT (pravibismane) is non-toxic to in vitro differentiated human lung epithelium at >50-fold higher than efficacious concentration against *P. aeruginosa* in sputum [see activity in CF sputum Fig. 1].

Figure 8: Effect of BisEDT in solution on cytotoxicity (A) and tissue integrity (B) after 1, 8, 24, and 48hr exposure

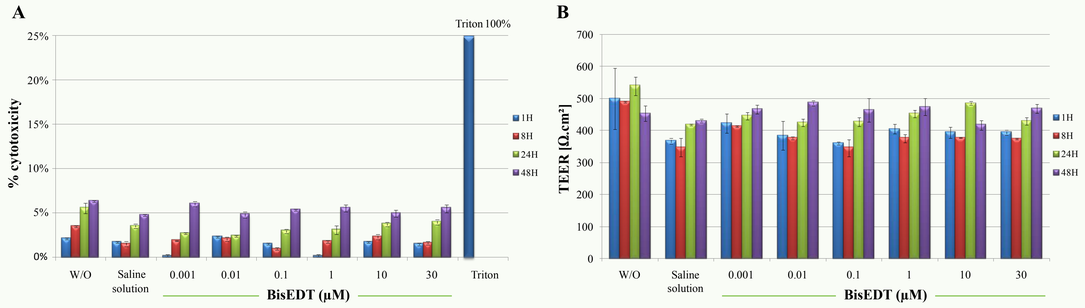
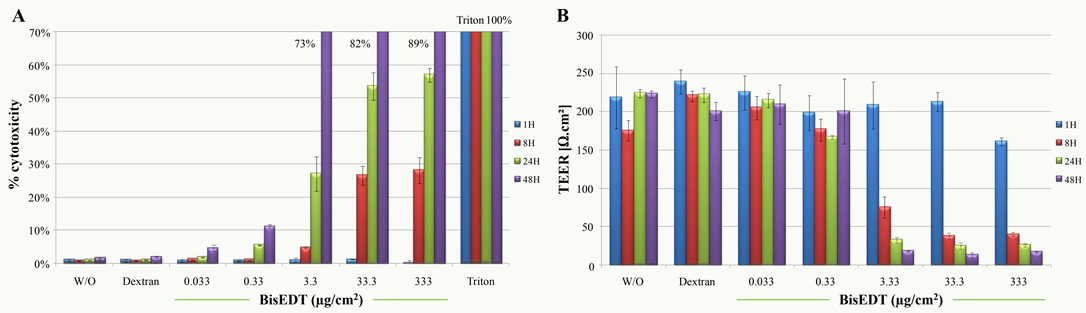


Figure 9: Effect of BisEDT applied as microtablets on cytotoxicity (A) and tissue integrity (B) after 1, 8, 24, and 48hr exposure

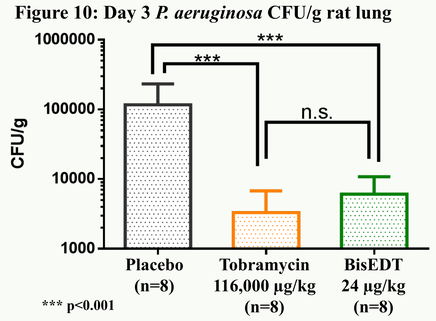


IN VIVO EFFICACY IN *P. AERUGINOSA* AGAR BEAD RAT MODEL

Procedure[2]:

- *P. aeruginosa* (patient isolated strain PA103) cultured agar was isolated as beads between 70 and 150 µm in diameter
- Rats were treated via nebulized aerosol inhalation with:
  - BisEDT: QD D0 and D2 - total 24 µg/kg lung deposited, or
  - Tobramycin positive control: BID D1 and D2 - total 116,000 µg/kg lung deposited, or
  - Placebo (saline) negative control
- Rats were sacrificed on D3 and lung homogenates were plated on media to quantify CFU/g lung tissue

Figure 10: Day 3 *P. aeruginosa* CFU/g rat lung



*** p<0.001

Results:

- BisEDT reduced *P. aeruginosa* burden relative to the negative control by approximately 1.5 log (p<0.001) [Fig. 10]
- In additional in vivo studies (data not shown), BisEDT (pravibismane) was shown to have a long lung residence time after inhalation delivery to rats ($t_{1/2}$ = 100 hrs) which is anticipated to provide long residual activity and provide favorable dosing schedule flexibility.

The efficacy of the BisEDT (pravibismane) group is similar in magnitude to the tobramycin positive control group despite the over 4000-fold higher total drug dose for the tobramycin group.

[2] Starke, JR et al. Pediatr Res. 1987; 22:698-702

ACTIVITY IN CF-PATIENT SPUTUM

Procedure[1]:

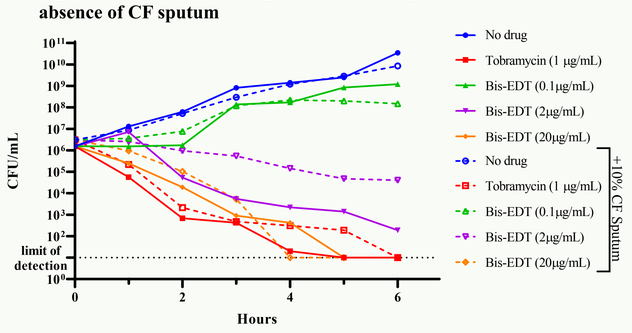
- Sputum was collected from CF patient volunteers and sterilized by UV irradiation.
- BisEDT or tobramycin was added to cation-adjusted Mueller-Hinton broth with or without 10% CF sputum inoculated with *P. aeruginosa* strain Pa01.
- Cultures were incubated at 37°C and aliquots were removed hourly for quantitation of CFU by serial dilution and plating on tryptic soy agar for 6 hours.

Results:

- Growth of Pa01 was not obviously inhibited or enhanced by the addition of sterile patient sputum alone.
- Sputum partially inhibited the activity of tobramycin, with approximately 0.5-1 log CFU/mL higher at most time points in cultures with sputum compared to without (tobramycin without sputum data not shown).

The activity of BisEDT (pravibismane) is partially inhibited in the presence of CF sputum but can be overcome by increasing concentration [Fig. 1].

Figure 1: BisEDT activity against Pa01 in the presence or absence of CF sputum



[1] King, P et al. Antimicrob Agents Chemother, 54:143-8, 2010.

CONCLUSIONS

- **BisEDT (pravibismane) offers strong potential as an inhaled antimicrobial/antibiofilm agent for suppression and treatment of multiple CF and NTM lung pathogens, including MDR *P. aeruginosa* and *M. abscessus* complex.**
- **BisEDT (pravibismane) has been formulated to provide delivery of efficacious doses by nebulization to both the central and peripheral lung.**

FUTURE DIRECTIONS

- **Based on the favorable delivery properties, and safety and efficacy of inhaled BisEDT (pravibismane) that have been demonstrated in the in vitro and in vivo studies conducted to date, GLP-toxicology studies in rats and dogs will be initiated, followed by Phase 1 clinical studies in healthy volunteers and CF patients with pulmonary infections.**

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