Poster # WMF21-0312

The Novel, Non-β-lactam PBP Inhibitor, ETX0462, Has Potent Activity Against Biothreat Pathogens *In Vitro* and *In Vivo*

CARB-X
Combating Antibiotic-Resistant Bacteria

ENTASIS THERAPEUTICS

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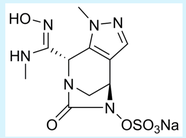
Introduction

ETX0462 is a novel, rationally designed, non-β-lactam diazabicyclooctane (DBO) covalent inhibitor of multiple penicillin-binding proteins (PBPs) with potent antibacterial activity, low frequencies of resistance, and *in vivo* efficacy against Gram-negative pathogens. We evaluated the *in vitro* antibacterial activity of ETX0462 against the biothreat pathogens *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Burkholderia mallei and B. pseudomallei. In vivo* efficacy of ETX0462 in Post-exposure Prophylaxis (PEP) and Delayed Treatment (DT) murine models of melioidosis and plague were determined against *B. pseudomallei* and *Y. pestis*, respectively.



ETX0462

Methods

Minimal inhibitory concentrations (MICs) were determined in a BSL-3 facility according to CLSI guidelines. *In vivo* PEP and DT models vs. *Y. pestis* CO92 (MIC = 0.25 mg/L) in BALB/c mice were performed via aerosol inhalation and intranasal delivery, respectively, at an inoculum of ~10^6 CFU/mouse (20X LD50). Ceftazidime and ciprofloxacin were positive controls. Drugs were administered 24 h post-infection (hpi) (PEP model) and 24, 36, and 42 hpi (DT model) for 4-5 days. In the DT model, half of the animals were sacrificed at 72 hpi (approximate time of death of vehicle controls) and bacterial burden was assessed in lungs and spleens. Survival was monitored for 21 days (PEP model) and 30 days (DT model) post-challenge, with bacterial burden assessed at sacrifice. Plasma ETX0462 pharmacokinetics were measured in a separate infected cohort upon treatment with 250 mg/kg of ETX0462.

In vitro Results

In vitro activity of ETX0462 as determined by MIC susceptibility against a select panel of biothreat pathogens are summarized in Tables 1 and 2.

ETX0462 demonstrated potent activity with MICs ≤ 2 mg/L against all pathogens studied.

Table 1: Susceptibility of a select panel of biothreat pathogens vs. ETX0462 or doxycycline.

		MIC (mg/L)	
Genus/species	Strain	ETX0462	Doxycycline
F.tularensis	Schu4 S4	0.125	0.5
F.tularensis	WY96-3418	0.125	0.5
F.tularensis	MA00-2987	0.125	0.5
F.tularensis	KY99-3387	0.25	0.5
F.tularensis	OR96-0246	0.125	0.5
Y. pestis	ZE94-2122	0.25	2
Y. pestis	PEXU2	0.25	1
Y. pestis	PB6	0.25	2
Y. pestis	Nepal516	0.25	1
B. anthracis	Graves	0.5	0.0313
B. anthracis	46-PY-5	0.5	0.0313
B. anthracis	Ames	0.5	0.0313
B. anthracis	Kruger B (A0442)	1	<0.0156
B. anthracis	Vollum (A0488)	0.5	0.0313
B. anthracis	WNA	0.5	0.0313
B. anthracis	A0318	0.5	0.0313
B. anthracis	A0471	0.5	0.0313
B. anthracis	ASC 506	0.5	0.0313
B. anthracis	ASC 525	1	0.0625
B. anthracis	2000032823 (CDC #1)	1	<0.0156
B. anthracis	2002734753 (CDC #2)	0.5	0.0313
B. anthracis	2010719149 (CDC #3)	0.5	0.0313
B. anthracis	2006200760 (CDC #4)	0.5	0.0625
B. anthracis	ASC 32	0.5	0.0313
B. anthracis	ASC 149	0.5	0.0313

Table 2: Susceptibility of a select panel of *Burkholderia spp.* vs. ETX0462 or ceftazidime.

		MIC (mg/L)	
Genus/species	Strain	ETX0462	Ceftazidime
B. pseudomallei	1106b	0.5	4
B. pseudomallei	1710a	0.5	8
B. pseudomallei	1710b	0.5	4
B. pseudomallei	406e	0.25	2
B. pseudomallei	MSHR435	2	8
B. pseudomallei	MSHR668	1	8
B. pseudomallei	MSHR465a	0.5	2
B. pseudomallei	NCTC 6700	0.5	4
B. pseudomallei	NCTC 7383	0.25	4
B. pseudomallei	NCTC 7431	0.5	4
B. pseudomallei	NCTC 10274	0.25	4
B. pseudomallei	NCTC 10276	0.5	2
B. pseudomallei	China 3 (MP-H, NBL 104)	0.25	1
B. mallei	China 7 (NBL7)	0.25	1
B. mallei	GB8 Horse 4	0.25	2
B. mallei	NCTC 120	0.5	4
B. mallei	NCTC 10248	0.25	1
B. mallei	11 (NCTC 10260)	0.5	4
B. mallei	China 7 (NCTC 12938)	0.25	1

In vivo Results: *Y. pestis*

All *in vivo* experimental procedures adhered to the guidelines detailed in *the Guide for the Care and Use of Laboratory Animals.*

ETX0462 demonstrated robust *in vivo* activity in a post exposure prophylaxis model of plague (*Y. pestis* CO92, Figure 1).
Similar mean survivorship was observed relative to positive controls ciprofloxacin and ceftazidime.
All treatment regimens were statistically significant relative to non-treatment controls that expired within 5 days of bacterial challenge.
Similar positive results were observed with delayed treatment out to 36h post infection (Figure 2).

Figure 1: Kaplan-Meier survival plot of *Y. pestis* CO92 in a Post-exposure Prophylaxis (PEP) model using an aerosolized dose (20 X LD_{50} of 6.8 x 10^4 CFU) following treatment with ETX0462, ciprofloxacin (CIP), or ceftazidime (CTZ), vs. vehicle control. All three agents show excellent *in vivo* efficacy relative to vehicle control (Log-rank test p <0.0001 vs. control).

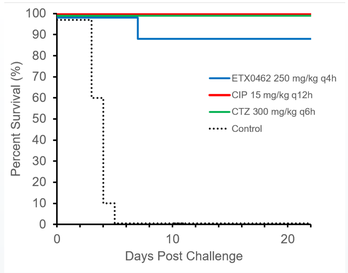
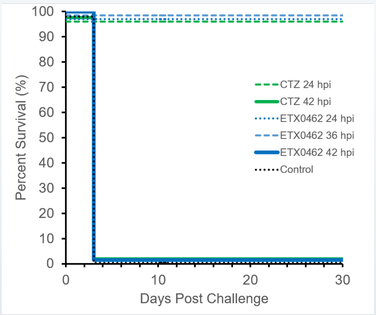


Figure 2: Kaplan-Meier survival plot of *Y. pestis* CO92 infected mice in a Delayed Treatment model using an intranasal challenge of 0.85 x 10^6 CFU following treatment with ETX0462 or ceftazidime (CTZ) vs. vehicle control



In vivo Results: *B. pseudomallei*

ETX0462 showed compelling activity in a melioidosis model utilizing a delayed treatment regimen of ETX0462 and ceftazidime as a comparator control (Figure 3).

All regimens of ETX0462 were superior and statistically distinct from ceftazidime regimens out to 24 hours post infection (hpi).
No bacterial CFU counts were observed in the tissues of surviving mice dosed with ETX0462 at the end of the study (Day 25, Figure 4).

Figure 3: Kaplan-Meier survival plot of *B. pseudomallei* MSHR435 infected mice following delayed treatment (2, 8 and 24 hours post-infection) with ceftazidime (CTZ) or ETX0462 vs. vehicle control. Mean survival for ETX0462 treatment groups were significantly different compared to ceftazidime treated groups (Log-rank test, p<0.01).

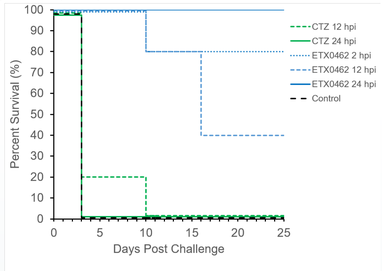
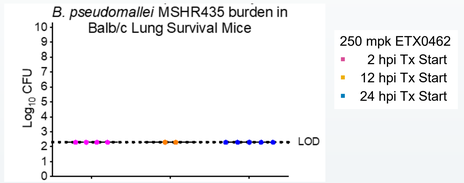


Figure 4. Lung CFU burden remaining in surviving animals (Day 25) of ETX0462 treatment arms (Limit of detection = 2.3 $\log_{10}$CFU/gm).



Summary and Conclusions

ETX0462 showed potent, broad-spectrum antibacterial activity *in vitro* against all biothreat pathogens tested.

Robust *in vivo* efficacy in two models of *Y. pestis* and *B. pseudomallei* infection warrants further investigation of efficacy vs. other biothreat pathogens. The efficacy of ETX0462 was superior to the ceftazidime control.

ETX0462 represents a novel class of antibiotic for the treatment of infections caused by Gram-negative and biothreat pathogens

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