Poster # WMF21-0292

Evaluation of the Pharmacokinetics/pharmacodynamics (PK/PD) of the Novel Diazabicyclooctane (DBO) ETX0462 Against *Pseudomonas aeruginosa* Infections, Including MDR Strains

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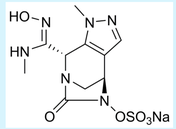
Introduction

ETX0462 is a 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. PK/PD driver determination and exposure response studies were carried out *in vitro* and *in vivo* utilizing a hollow-fiber infection model (HFIM) and murine neutropenic thigh and lung infection models against contemporary *P. aeruginosa* isolates with minimal inhibitory concentrations (MICs) ranging from 0.25 to 4 mg/L..



ETX0462

Methods

ETX0462 dose fractionation experiments were performed *in vitro* in a hollow-fiber infection model (HFIM) against six *P. aeruginosa* isolates with minimal inhibitory concentrations (MICs) ranging from 0.25 to 4 mg/L (Table 1). Daily doses were administered via a 2-hour infusion divided into q6h, q12h, and q24h regimens over a 64-fold concentration range. Exposure-response was evaluated using Hill-type model fitting of the change in log10 CFU/mL over 24 hours vs. AUC/MIC, Cmax/MIC, and %Time>MIC. Goodness-of-fit parameters R2 and weighted sum of squared residuals were used to determine the PK/PD index most closely correlated with antibacterial activity. *In vivo* dose response studies with 7 strains were performed in murine neutropenic thigh and lung models with supporting PK. Drug concentrations of ETX0462 were determined by LC/MS/MS. Emax fitting of unbound ETX0462 %Time>MIC vs. 24-hour change in log_{10} CFU/g of tissue were used to establish PK/PD magnitudes for stasis, 1-log_{10} kill, and 2-log_{10} kill.

Table 1. Genome sequencing and MIC summary of *P. aeruginosa* strains.

P. aeruginosa Strain ID	Whole Genome Sequencing Results	MIC (mg/L)
ARC6347	PDC-24+; OXA-486+; OpdR [E181K]; MexA [L338P]; MexT [G79fs];	0.25
ARC3514	PDC-19a+; OXA-488+; KPC-2+; PBP4 (DacB) [G287S]; OprD [W277*]; MexT [G79fs];	4
ARC3727	PDC-3+; OXA-4+; OXA-486+; PC1+; PBP3 [F533L]; OprD [K389fs]; MexT [G79fs];	0.5
ATCC 27853	PDC-5+; OXA-396+; MexT [G79fs];	0.5
ARC3856	PDC-19a; OXA-488+; GES-7+; MexR [R85L]; MexT [G79fs]; MexZ [V43G];	4
ARC3505	PDC-5+; OXA-10+; OXA-494+; PER-1+; MexT [G79fs]; MexZ [E98fs];	2
ARC3506	PDC-35+; OXA-10+; OXA-488+; VEB-1+; OprD [P405fs]; MexT [G79fs]; MexZ [G169fs];	2

Results: *In vitro* Hollow Fiber PK/PD Driver Determination

- Composite Emax fitting of ETX0462 PK/PD index vs. 24h change in CFU burden are shown in Figures 1, 2, and 3 for %T>MIC, Cmax/MIC, and AUC/MIC, respectively.
- %T>MIC was the most correlated index to observed activity across the 6 strains evaluated in the hollow-fiber infection model (ARC6347, ARC3514, ARC3727, ARC3856, ARC3505, and ARC3506, R^2 = 0.89).
- Individual strain PK/PD driver analysis demonstrated %T>MIC to be the most correlated to observed activity. (data not shown).

Figure 1. Emax fitting of ETX0462 %Time>MIC vs 24h change in CFU burden.

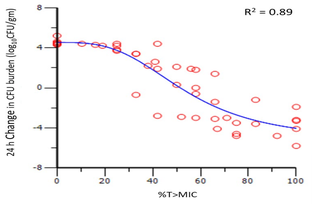


Figure 2. Emax fitting of ETX0462 Cmax/MIC vs 24h change in CFU burden.

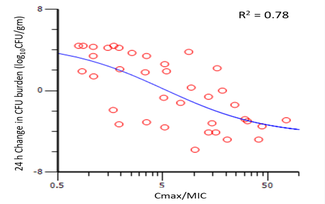
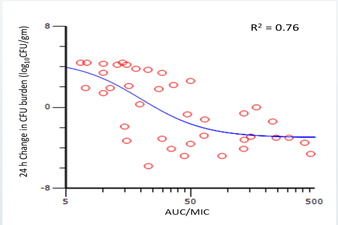


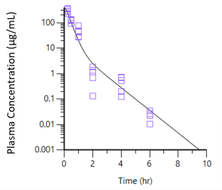
Figure 3. Emax fitting of ETX0462 AUC/MIC vs 24h change in CFU burden.



Results: *In vivo* Murine PK and Neutropenic Infection Models

- All animal work was performed in accordance to the Animal Welfare Regulatory Act (9CFR3) using IACUC approved protocols revewed by OLAW and completed under the supervision of an attending veterinarian.
- Plasma PK was completed in CD-1 mice following single subcutaneous doses of 10, 50 and 250 mg/kg and fitted using a two compartment PK model (Figure 4).
- PK parameter estimates were used to interpolate exposures across the dose range used in the lung and thigh infection models and determine f%T>MIC
- The 24h change in CFU burden for each dose regimen used in thigh and lung models are summarized in Tables 2 and 3, respectively.
- Free %T>MIC plotted with CFU burden for each regimen at 24h post initiation of dose is depicted in Figure 5 vs. ARC6347.

Figure 4. Two-compartment PK fitting of a 250 mg/kg subcutaneous dose of ETX0462 in mice.



Parameter	Units	Estimate
V/F	mL/kg	500
K01	1/hr	25
CL/F	mL/hr/kg	1700
V2/F	mL/kg	90
CLD2/F	mL/hr/kg	100

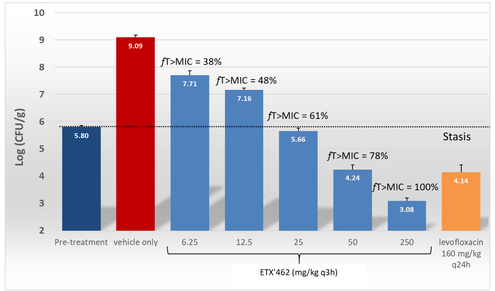
Table 2. Change in CFU burden in thigh tissue following 24h (q3h) administration of ETX0462.

	Change in CFU/gm at 24 hour post initiation of therapy Thigh model/ PA strain (MIC)						
Dose	ARC6347	ARC3514	ARC3727	ATCC 27853	ARC3856	ARC3505	ARC3506
(mg/kg)	(0.25 mg/L)	(4 mg/L)	(0.5 mg/L)	(0.5 mg/L)	(4 mg/L)	(2 mg/L)	(2 mg/L)
0 (vehicle)	3.24	2.57	3.93	3.14	2	3.14	1.76
6.25	1.91	3.05	1.82	1.56	1.61	2.83	0.72
12.5	1.36	2.79	1.81	1	1.27	1.77	-0.05
25	-0.14	--	--	--	--	--	--
50	-1.56	2.25	-1.44	-2.17	0.01	0.67	-0.54
200	-2.72 (250 mg/kg)	-1.65	-2.1	-2.17	-2.55	-2.02	-1.37

Table 3. Change in CFU burden in lung tissue following 24h (q3h) administration of ETX0462.

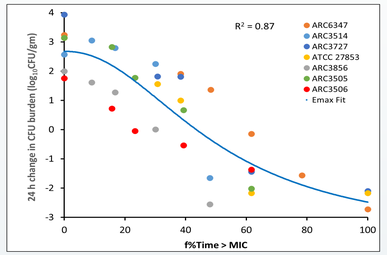
	Change in CFU/gm at 24 hour post initiation of therapy Lung model/PA strain (MIC)		
Dose	ARC3727	ATCC 27853	ARC3506
(mg/kg)	(0.5 mg/L)	(0.5 mg/L)	(2 mg/L)
0 (vehicle)	2.94	2.85	2.6
6.25	-0.18	-2.11	-1.32
12.5	-1.67	-2.13	-2.61
25	-1.83	-3.74	-2.96
50	-1.84	-3.58	-3.36
200	-2.78	-3.96	-3.3

Figure 5. CFU burden vs. ETX0462 dose regimen with f%T>MIC estimates vs. ARC6347



Composite Emax Fitting and Time above MIC Magnitude Summary

- The 24h change in CFU burden vs. f%T>MIC data was combined across all 7 strains evaluated in the neutropenic thigh model and fit to an Emax model (Figure 6).
- Free %T>MIC requirements to achieve stasis, 1-log_{10} kill, and 2-log_{10} kill, are summarized in Table 4.
- Exposure magnitude requirements to achieve PK/PD endpoints *in vivo* were generally equivalent to results from the *in vitro* hollow-fiber model.



PK/PD Endpoint	N strains*	Percent T>MIC (%)
In vitro hollow-fiber		
stasis	6	50.2
1-log kill	6	57.1
2-log kill	6	67.1
In vivo neutropenic thigh		
stasis	7	43.2
1-log kill	7	58.6
2-log kill	7	81.6

*composite Emax model fitting of *P.aeruginosa* strains (MIC range 0.25 - 4 mg/L)

Summary and Conclusions

- The antibacterial activity of ETX0462 was time-dependent, with the PK/PD index of %Time>MIC most closely correlated with activity.
- PK/PD endpoint magnitudes were similar *in vitro* and *in vivo*, with 1-log_{10} kill achieved when unbound concentrations of ETX0462 exceeded the MIC for 60% of the dosing interval.
- These results may be useful for establishing clinical doses and support further development of ETX0462 for the treatment of Gram-negative infections.

Presented at World Microbe Forum 2021