Evaluation of Pharmacokinetics/Pharmacodynamics of the Novel β-lactamase Inhibitor, ETX2514, in Combination with Sulbactam Against *Acinetobacter baumannii*

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ABSTRACT

Background: ETX2514 is a novel β-lactamase inhibitor with broad spectrum activity against Ambler class A, C, and D serine β -lactamases that successfully restores activity of sulbactam (SUL) against *Acinetobacter baumannii*. In the study described herein, the PK/PD index associated with ETX2514 efficacy and the magnitude of this PK/PD index required for different levels of bacterial reduction were evaluated using chemostat and/or neutropenic murine thigh infection models.

Methods: Infusion regimens of ETX2514 in combination with SUL were completed in the chemostat model over 24 h. Total daily doses of ETX2514 were fractionated into regimens administered q6h, q12h, and q24h to determine PK/PD index associated with efficacy based on the evaluation of an *A. baumannii* MDR strain (ARC5081 SUL/ETX2514 Mean MIC = 2.9 mg/L). Three additional MDR strains with SUL/ETX2514 MICs of 4-8 mg/L were completed in both the chemostat and neutropenic murine-thigh infection models with exposure for SUL equivalent to a clinical dose of 1 g q4h and ETX2514 ranging from 0.125 to 1.0 g q4h. Serial CFU samples from both *in vitro* and *in vivo* models were counted and the relationships between change in $\log_{10}$ CFU from baseline at 24 h and AUC:MIC ratio, C_{max}:MIC ratio and %T>ETX2514 concentrations (C_T) were evaluated using Hill-type models.

Results: *In vitro* infection model results showed that %Time>C_T of 0.75 mg/L in the presence of SUL was the PK/PD index predictive of ETX2514 efficacy; the r^2 for the relationship between change in $\log_{10}$ CFU from baseline at 24 hours and %Time>C_T of 0.75 mg/L was 0.83. Normalizing the data to dosing interval (C_{avg} vs. change in $\log_{10}$ CFU) suggested time dependency. However, AUC:MIC ratio was also considered a viable PK/PD index in a setting of q6h dose administration. *In vivo* infection model results showed that AUC:MIC ratio and T>C_T of 0.75 mg/L were both highly correlated with ETX2514 activity, with AUC:MIC targets of 10 and 30 for net bacterial stasis and 1-$\log_{10}$ CFU reduction, respectively. These PK/PD targets agreed with those determined using the chemostat model. Regimens utilizing drug exposures equivalent to a clinical dose of 1 g SUL + 1 g ETX2514 q6h demonstrated > 1-$\log_{10}$ CFU reduction *in vivo* against MDR strains with MICs up to 8 mg/L. Efficacy against the MDR strain with an MIC of 8 mg/L, however, was not observed in the chemostat model.

Conclusions: The combination of ETX2514 and SUL demonstrated *in vitro* and *in vivo* efficacy against clinical *A. baumannii* strains with MICs up to 4 mg/L. The results of these studies will help to guide future dose selection.

INTRODUCTION

- *Acinetobacter baumannii* is a Gram-negative pathogen known to cause severe infections associated with high mortality and morbidity. The majority of clinical isolates are classified as either multi-drug resistant (MDR), or extensively drug resistant.
- Sulbactam is a β-lactamase inhibitor (BLI) that demonstrates intrinsic activity against *A. baumannii.* The widespread prevalence of β-lactamases, however, has now limited the utility of sulbactam. While Class D β-lactamases are quite common in *A. baumannii*, Class A and Class C are also often co-expressed in these pathogens, necessitating the need for a broad-spectrum BLI capable of inhibiting Class A, C, and D β-lactamases.
- ETX2514 is a potent inhibitor of Ambler Class A, C, and D β-lactamases. Previous studies have demonstrated that sulbactam efficacy is restored in the presence of ETX2514 when sulbactam exposure is targeted at 50% Time>MIC [1].
- The combination of ETX2514/sulbactam (ETX2514SUL) is being developed to treat infections caused by MDR *A. baumannii*.
- The studies described herein seek to define the PK-PD index and exposure requirements of ETX2514 using an *in vitro* chemostat model as well as *in vivo* dose response studies completed in a neutropenic murine-thigh infection model.


ETX2514


Sulbactam

METHODS

***In Vitro* Minimum Inhibitory Concentration Testing**

- Broth microdilution MIC values were evaluated according to CLSI guidelines (M07-A10, 2015) using cation-adjusted Mueller-Hinton broth (MHBII, Sigma-Aldrich, St. Louis, MO). The MIC of ETX2514SUL was measured by serial dilution of sulbactam in the presence of a fixed concentration of 4 mg/L ETX2514.

***In Vitro* Chemostat Model**

- Bacterial colonies grown overnight on blood agar plates were inoculated in MHBII and incubated at 35°C until reaching log phase growth (~1 hour). Approximately 1 mL of bacteria (1 x 10^8 CFU/mL) was introduced to a central reservoir with 109 mL MHBII to dilute the bacterial culture and reach the target CFU (1 x 10^6 CFU/mL). After 5 minutes, each chemostat bottle was exposed to test articles via a syringe pump (New Era Syringe Pump, Farmingdale, NY).
- Fluctuating concentrations of sulbactam, representing unbound concentrations observed after a 2 g dose administered every 6 hours (q6h), were infused in combination with a range of projected ETX2514 doses via a 3-hour infusion.
- ETX2514 dose-fractionation studies were completed in order to identify the PK-PD index associated with ETX2514 efficacy.

METHODS

- ETX2514 exposures associated with total daily doses of 0.5, 2, 4, and 8 g were fractionated by the area under the concentration time curve over 24 hours ($AUC_{0\text{-}24}$) into regimens administered every 6, 12, and 24 hours, over a 3 hour infusion.
- The relationships between change in $\log_{10}$ CFU and the PK-PD indices were evaluated using Hill-type models and non-linear least squares regression.

In Vivo Mouse Infection Model

- Mice were rendered neutropenic and infected in each thigh with *A. baumannii* (~6 $\log_{10}$ CFU/thigh) using established models [2]. Infected mice were treated via subcutaneous administration 2 hours post inoculation with either sulbactam alone, ETX2514 alone, or sulbactam in the presence of ETX2514 at doses of 1.25 to 200 mg/kg every 3 hours (q3h).
- For each strain, doses of sulbactam were set at 15, 75, or 150 mg/kg in order to maintain unbound concentrations above the MIC values of 0.64, 4, and 8 mg/L, respectively, for 50% of the dosing interval. Efficacy was determined as the change in viable bacterial counts in tissue 24 hours after start of treatment. The relationships between change in $\log_{10}$ CFU from baseline and the PK-PD indices were evaluated using Hill-type models and non-linear least squares regression.

RESULTS

Table 1. Mean MIC values and β-lactamase content of *A. baumannii* strains

A. baumannii strain	β-Lactamase content	Mean MIC value (mg/L)			Number of MIC runs
		ETX2514	Sulbactam	Sulbactam + ETX2514 at 4 mg/L	
ARC3486	TEM-1; OXA-66; OXA-72	>32	32	0.64	12
ARC5077	OXA-72	>32	8	4.0	11
ARC5081	OXA-23; OXA-94	32	16	2.9	13
ARC5950	OXA-23; OXA-10/69	>32	>32	8.0	11
ARC5955	TEM-1; OXA-23; OXA-66; ADC-82	>32	>32	4.0	3

Figure 1. $\log_{10}$ CFU/mL versus time following q6h administration of sulbactam and ETX2514

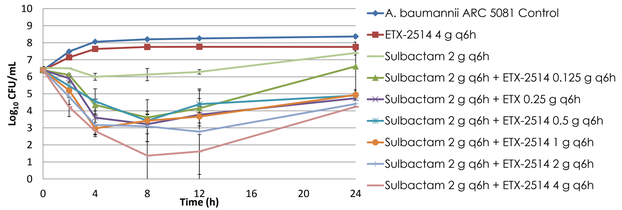
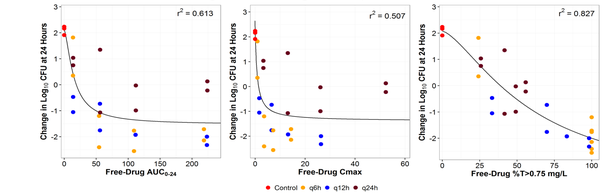


Figure 2. Relationship between change in $\log_{10}$ CFU at 24 h and ETX2514 AUC, maximum concentration (C_{max}), or %Time > ETX2514 concentration threshold of 0.75 mg/L following dose fractionation studies, with dosing intervals designated by color



RESULTS

Table 2. ETX2514 dose-fractionation vs. *A. baumannii* ARC5081 in the *in vitro* chemostat model

ETX2514 exposure	r^2	Stasis	1-$\log_{10}$ Reduction	2-$\log_{10}$ Reduction
$AUC_{0\text{-}24}$ (mg•h/L)	0.61	18.9	49.8	-
C_{max}	0.51	N/D	N/D	N/D
% T>0.75 mg/L	0.83	38.9%	62.2%	100%

N/D = Not Determined

Figure 3. Normalizing the ETX2514 AUC by TAU (dosing interval) suggests a minimal increase in activity beyond 120 mg•h/L or AUC/MIC of ~40 vs. ARC5081 in the *in vitro* chemostat model

ARC5081 (MIC=2.9 mg/L)

ETX2514 exposure	Stasis	1-log kill	2-log kill
$AUC_{0\text{-}24}$ (mg•h/L)	19	50	–
AUC/MIC	6.5	17	–

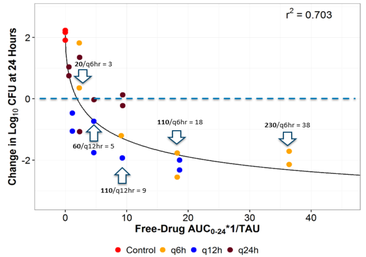


Figure 4. ETX2514 AUC correlates to *in vivo* efficacy against ARC3486 and ARC5081 in the neutropenic murine-thigh infection model

ARC3486 (MIC=0.64 mg/L)

ETX2514 exposure	Stasis	1-log kill	2-log kill
$AUC_{0\text{-}24h}$ (mg•h/L)	6.1	22	67
AUC/MIC	9.5	34	105

ARC5081 (MIC=2.9 mg/L)

Exposure	Stasis	1-log kill	2-log kill
$AUC_{0\text{-}24h}$ (mg•h/L)	12	38	101
AUC/MIC	4.1	13	35

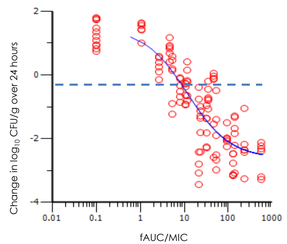


Table 3. Clinical Phase 1 exposure of ETX2514 in healthy volunteers relative to PK-PD targets [3]

Dose	ETX2514 $AUC_{0\text{-}24h}$ (mg•h/L)	*f*%T>0.75 mg/L[†]	*f*AUC/MIC[†] (MIC 4 mg/L)
1.0 g q6h	216	100%	49
0.5 g q6h	108	100%	24
0.25 g q6h	55	83%	12
0.125 g q6h	28	60%	6.3

[†]Unbound exposure; fraction unbound = 0.9; MIC breakpoint target of 4 mg/L considered

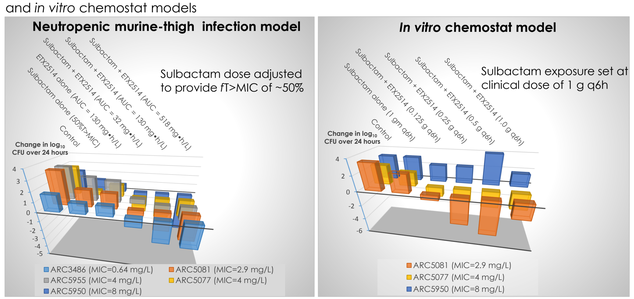
RESULTS

Table 4. Exposure of sulbactam and ETX2514 in the *in vivo* murine and *in vitro* chemostat models

Drug	Dose	*f*$AUC_{0\text{-}24}$ (mg•h/L)[‡]
Murine studies		
Sulbactam	150 mg/kg q3h	467
	75 mg/kg q3h	234
	15 mg/kg q3h	46
ETX2514	12.5 mg/kg q3h	32
	50 mg/kg q3h	130
	200 mg/kg q3h	518
Chemostat studies		
Sulbactam	1 g q6h	196
ETX2514	0.125 g q6h	29
	1 g q6h	248

[‡]Unbound exposure; fraction unbound = 1.0 for both sulbactam and ETX2514 in mouse plasma

Figure 5. Exposure response of Acinetobacter strains to sulbactam and ETX2514 in *in vivo* murine and *in vitro* chemostat models



CONCLUSIONS

- Dose-fractionation studies for ETX2514 completed using the *in vitro* chemostat model in the presence of sulbactam (2 g q6h target) suggested that %T> ETX2514 concentration of 0.75 mg/L was highly correlated with efficacy.
- Results of the dose-fractionation *in vitro* chemostat model studies suggested that ETX2514 AUC normalized by TAU may also be a useful exposure target for supporting dose selection.
- Results of the *in vivo* studies demonstrated that ETX2514 AUC/MIC was highly correlated with efficacy.
- Results from *in vitro* chemostat dose-response studies evaluating clinical doses of sulbactam and ETX2514 suggest efficacy could be achieved against isolates with ETX2514 potentiated sulbactam MIC values up to and including 4mg/L.

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