LB-117

In Vitro and *In Vivo* Efficacy of the Novel β-lactamase Inhibitor ETX2514 Combined with Sulbactam Against Multidrug Resistant *Acinetobacter baumannii*

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Abstract

Background: The novel diazabicyclooctenone β-lactamase inhibitor ETX2514 is active against Class A, C and D serine β-lactamases and effectively restores the antibacterial activity of sulbactam against multidrug resistant (MDR) *A. baumannii*. To further evaluate the therapeutic potential of this combination, its PK/PD drivers *in vitro* and *in vivo* were assessed.

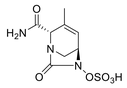
Methods: The *in vitro* activity of ETX2514-sulbactam was characterized using an *in vitro* hollow-fiber model. Dose range and dose fractionation experiments varying concentration (C_{max}, AUC) and frequency of administration of ETX2514 and sulbactam (q3h, q6h, q12h, and q24h) were used to determine PK/PD endpoints against five clinical MDR *A. baumannii* strains. The same strains were evaluated *in vivo* by bacterial burden reduction in a tissue-based abscess in neutropenic thigh and lung infection models to validate exposure targets established *in vitro*.

Results: Initial results of *in vitro* and *in vivo* investigations with sulbactam alone vs. a sulbactam-sensitive *A. baumannii* isolate (MIC = 2 μg/mL) suggested Time > MIC as the PK/PD driver with 21 to 47% Time > MIC associated with > 1-log kill *in vivo*. Against MDR strains (with sulbactam MICs > 8 μg/mL), ETX2514 restored MIC potency to < 2 μg/mL and demonstrated a PK/PD driver of Time > critical threshold (C_T) in the hollow-fiber model system. E_{max} modelling of *in vivo* data from thigh and lung models suggested ETX2514 effectively restores sulbactam activity, and 1-log to 2-log kill is realized with Time > MIC for the combination ranging from 26 to 55% of the dosing interval.

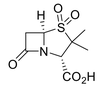
Conclusions: The combination of ETX2514 and sulbactam is highly effective against clinical *A. baumannii* strains *in vitro* and *in vivo*. These results support further evaluation of this combination to treat MDR *A. baumannii* infections.

Introduction

Multi-drug resistant (MDR) *A. baumannii* has become a significant public health concern and is now classified as a serious threat pathogen in the recent CDC's "Antibiotic Resistance Threats" report. The pathogen has been implicated in serious infections including pneumonia/ventilator-associated pneumonia (VAP), urinary tract (UTI), bloodstream, and wound infections. With few treatment options available due to lack of efficacy or toxicity, new agents are needed to address the rising rates of mortality associated with *A. baumannii* infections. The novel diazabicyclooctenone β-lactamase inhibitor ETX2514 is active against a broad range of Class A, C and D serine β-lactamases and effectively restores the antibacterial activity of sulbactam against multidrug resistant (MDR) *A. baumannii.* To further evaluate the therapeutic potential of this combination, its *in vitro* activity and PK/PD drivers were determined using a hollow-fiber infection model. *In vivo* efficacy was confirmed using murine thigh and lung models.



ETX2514



sulbactam

Methods

In vitro Hollow-fiber: Steady state fluctuating free plasma drug concentrations were simulated in the *in vitro* hollow-fiber infection model (Blaser, 1987; Tam, 2007) to evaluate bacterial response to various sulbactam and ETX2514 exposures over a period of 24 h. Approximately 15 mL of bacteria (inoculum ~10^6 CFU/mL) were grown in polysulfone hollow fiber cartridges and were exposed to various dosing regimens of sulbactam and ETX2514. Serial samples were collected to determine CFUs and drug concentration. Different dosing regimens of sulbactam and ETX2514 (q24h, q12h, or q6h) were administered to investigate the PK/PD index. A one compartment model was fit to the observed data using Phoenix 6.2.0. Change in bacterial burden at 24 h relative to burden at time zero were correlated to drug exposures. A sigmoidal dose-response (E_{max}) model was used to analyze the PK/PD index.

In Vivo Mouse Infection Models: Mice were rendered neutropenic with cyclophosphamide and infected with *A. baumannii* for lung and thigh models (Craig 1996, Gerber 1983). Infected mice were treated via subcutaneous administration 2 hours post bacterial inoculation with either sulbactam alone or sulbactam in the presence of ETX2514 at a constant 4:1 ratio, or as a 15 mg/kg dose of sulbactam with varied doses of ETX2514 administered q3h. Efficacy was determined as the change in viable bacterial counts in tissue 24 hours after start of treatment. Plasma exposures for both sulbactam alone and sulbactam-ETX2514 combinations were determined in a group of infected satellite mice and used in support of a population PK model to normalize the dose response to exposure-effect relationships. A sigmoidal E_{max} model was utilized to determine the magnitude requirements associated with 1-log and 2-log reductions for sulbactam in the presence of ETX2514, utilizing both neutropenic thigh and lung model models.

Results: *In Vitro* Hollow-Fiber

MICs (μg/mL) *of A. baumannii* strains utilized in PK/PD studies

Strain (β-Lactamase content)	Type	Meropenem	ETX2514	Sulbactam	Sulbactam + ETX2514 (4 μg/mL)
ARC2058 (OXA-95)	Susceptible	0.25	>32	2	1
ARC3484 (OXA-23, OXA-64, TEM-1)	MDR	>8	>32	16	0.5
ARC3486 (OXA-66, OXA-72, TEM-1)	MDR	>8	>32	32	0.5
ARC5079 (OXA-65, OXA-72)	MDR	>8	>32	32	1
ARC5081 (OXA-23, OXA-66)	MDR	>8	>32	8	2
ARC5091 (OXA-23, OXA-78)	MDR	>8	>32	8	1

Sulbactam *in vitro* PK/PD:

PK/PD driver determination of sulbactam vs. susceptible strain ARC2058 in the *in vitro* hollow-fiber model

PK/PD Index	EC_{80}±SE	%CV	R^2	WSSR
AUC	135 ± 86	63	0.75	79
C_{max}	12 ± 6	51	0.50	140
%Time>MIC	**90 ± 4**	**5**	**0.96**	**15**

EC_{80} ±SE = 80% effective concentration ± standard error %CV = coefficient of variance R^2 = correlation coefficient WSSR = weighted sum of the squared residuals

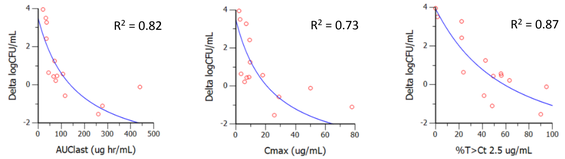
ETX2514 *in vitro* PK/PD:

ETX2514 dose fractionation experiments with sulbactam present to cover 80% Time > combination MIC were used to determine its PK/PD driver vs. MDR *A. baumannii* strains ARC5079 and ARC5081

PK/PD Index	ARC5079		ARC5081	
	R^2	WSSR	R^2	WSSR
AUC	0.85	38	0.82	13
C_{max}	0.83	24	0.73	19
Time >[C_T] (μg/mL)				
0.5	0.71	37	0.37	33
1	0.83	24	0.63	23
2.5	**0.87**	**18**	**0.87**	**10**
4	0.85	21	0.81	14

R^2 = correlation coefficient WSSR = weighted sum of the squared residuals

ETX2514 PK/PD driver determination vs MDR *A. baumannii* ARC5081



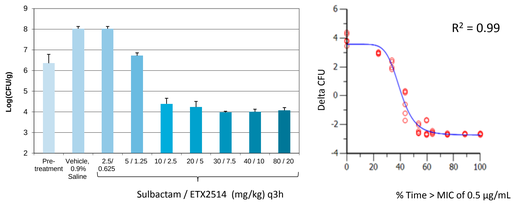
AUC and Time > a critical threshold (C_T) were highly correlated to observed *in vitro* activity of ETX2514 when administered in combination with sulbactam.

Concentrations below C_T = 2.5 μg/mL, however, appeared to be less dynamically linked to activity as suggested by poor correlation coefficients. Confirmation of driver and resolution of magnitude were studied further using *in vivo* murine thigh and lung infection models.

Results: *In Vivo* Thigh and Lung Studies

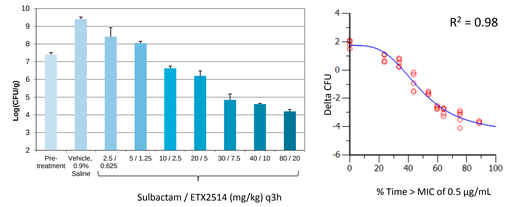
Sulbactam:ETX2514 dose response (4:1 dose ratio)

MDR *A. baumannii* ARC3486 thigh infection
(sulbactam MIC =>32 μg/mL, sulbactam:ETX2514 MIC = 0.5 μg/mL)



Sulbactam:ETX2514 dose response (4:1 dose ratio)

MDR *A. baumannii* ARC3486 lung infection
(sulbactam MIC =>32 μg/mL, sulbactam:ETX2514 MIC = 0.5 μg/mL)



Sulbactam:ETX2514 dose response summary (4:1 dose ratio)

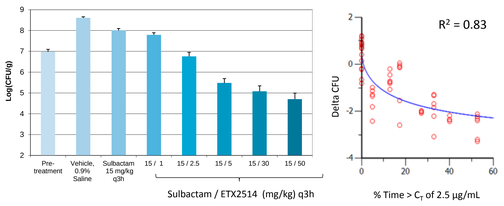
Strain	%T>MIC sulbactam in the presence of ETX2514	
	1-log reduction	2-log reduction
Thigh model:		
ARC3484	36.2 ± 0.6	40.2 ± 1.0
ARC3486	45.7 ± 0.7	52.9 ± 1.2
ARC5079	36.4 ± 0.7	42.0 ± 1.2
ARC5081	26.3 ± 0.7	28.8 ± 0.9
ARC5091	26.2 ± 0.3	29.3 ± 0.4
Lung model:		
ARC3486	45 ± 1	55 ± 1
ARC5079	42 ± 1	53 ± 1
ARC5081	37 ± 1	44 ± 1

Dosing sulbactam:ETX2514 at a 4:1 ratio effectively restored the activity of sulbactam with 2-log kill achieved when sulbactam concentrations exceeded the combination MIC (sulbactam MIC with 4 μg/mL ETX2514) for 50% of dosing interval. Data obtained in thigh is relatively consistent with lung.

These magnitudes are consistent with the predicted clinical exposures of sulbactam (30-50 %T>MIC) used to successfully treat *A. baumannii* infections

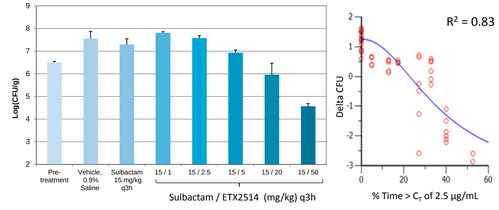
ETX2514 dose titration with 15 mg/kg of sulbactam (T>MIC of 40%)

MDR *A. baumannii* ARC3486 thigh infection
(sulbactam MIC =>32 μg/mL, sulbactam:ETX2514 MIC = 0.5 μg/mL)



ETX2514 dose titration with 15 mg/kg of sulbactam (T>MIC of 20%)

MDR *A. baumannii* ARC5079 thigh infection
(sulbactam MIC =>32 μg/mL, sulbactam:ETX2514 MIC = 1.0 μg/mL)



ETX2514 dose titration summary with 15 mg/kg sulbactam

Strain	%T>C_T of 2.5 μg/mL ETX2514 in the presence of sulbactam	
	1-log reduction	2-log reduction
ARC3486	8.2 ± 2.7	38.1 ± 9.4
ARC5079	35.5 ± 2.7	54.0 ± 12.3
ARC5081	25.2 ± 21.2	27.1 ± 2.9

Conclusions

• *In vitro* hollow fiber studies suggest PK/PD drivers of T>MIC and T>C_T for sulbactam and ETX2514, respectively.

• *In vivo* neutropenic thigh studies utilizing a 4:1 dosing ratio of sulbactam:ETX2514 suggests ETX2514 restores sulbactam susceptibility with 2-log kill achieved at 50% T>MIC in thigh and lung models.

• Dose titration of ETX2514 in the presence of sulbactam at 20-40% T>MIC suggests a 2-log kill can be achieved when concentrations of ETX2514 exceed a critical threshold of 2.5 μg/mL for 50% of the dosing interval.

• Sulbactam-ETX2514 demonstrates excellent *in vitro* and *in vivo* activity and represents a promising combination to treat MDR *A. baumannii* infections.

References

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