Poster #1844

Eravacycline is Active against Carbapenem-resistant *Enterobacteriaceae* and *Acinetobacter baumannii* Isolates in the FDA-CDC Antimicrobial Resistance Isolate Bank Panels

Corey Fyfe, Gabrielle LeBlanc, Joyce A. Sutcliffe, Trudy H. Grossman

Tetraphase Pharmaceuticals, Watertown, MA

TETRAPHASE PHARMACEUTICALS

Contact:
Tetraphase Pharmaceuticals Medical Information (TPMI)
medinfo@tphase.com
617-715-3600

Abstract

Background: Eravacycline (ERV) is a novel, fully-synthetic fluorocycline antibiotic of the tetracycline class with *in vitro* activity against Gram-negative pathogens, including extended-spectrum β-lactamase and carbapenemase-producing *Enterobacteriaceae* (ENT) as well as multidrug-resistant strains of *Acinetobacter baumannii*. To further characterize its activity *in vitro*, ERV was tested against new reference panels made available by the FDA and CDC for the testing of new antibacterial agents.

Methods: Using Clinical Laboratory Standards Institute methodology, minimal inhibitory concentration (MIC) values for antibiotics were determined for isolates in the FDA-CDC Antimicrobial Resistance Isolate Bank (AR Bank) panels: 1) ENT Carbapenem Breakpoint Panel ("Breakpoint"), 2) Gram Negative Carbapenemase Detection Panel ("Detection") and 3) ENT Carbapenemase Diversity Panel ("Diversity"). The Breakpoint panel contained 31 ENT of which 7 were meropenem-resistant, including 5 bla_{KPC} isolates. The Detection panel was comprised of 80 isolates total; a 52 ENT subset contained carbapenemase genes bla_{IMP} (n=1), bla_{KPC} (n=8), bla_{NDM} (n=10), bla_{OXA} (n=5), bla_{SME} (n=2), bla_{VIM} (n=3). The Diversity panel contained 53 ENT with carbapenemase genes bla_{IMI} (n=2), bla_{IMP} (n=1), bla_{KPC} (n=18), bla_{NDM} (n=20), bla_{OXA} (n=5), bla_{SME} (n=6), and bla_{VIM} (n=2). There were 14 *A. baumannii* isolates across all panels, of which 4 isolates had bla_{NDM} and 8 had bla_{OXA} genes.

Results: The ERV $MIC_{50/90}$ values and ranges against the Breakpoint, Detection (ENT subset), Diversity panels and *A. baumannii* isolates are shown in the Table.

FDA-CDC Panel	n	ERV MIC_{50} (μg/mL)	ERV MIC_{90} (μg/mL)	ERV Range (μg/mL)
Breakpoint	31	0.25	1	0.031-2
Detection (ENT subset)	52	0.5	2	0.031-4
Diversity	53	0.5	1	0.031-8
Acinetobacter baumannii	14	0.25	1	0.031-2

Conclusion: ERV maintained potency against carbapenem-resistant ENT and *A. baumannii* isolates in the AR Bank, including those containing carbapenemase genes prevalent in contemporary clinical isolates.

Background

Eravacycline (ERV) is a novel, fully-synthetic fluorocycline antibiotic of the tetracycline class in clinical development for treatment of intraabdominal and urinary tract infections, including those caused by multidrug-resistant Gram negative pathogens. Eravacycline has historically exhibited potent *in vitro* activity against Gram-negative pathogens, including extended-spectrum β-lactamase and carbapenemase-producing *Enterobacteriaceae* as well as multidrug-resistant strains of *Acinetobacter baumannii*. To further characterize this *in vitro* activity, ERV was tested against reference panels made available by the FDA and CDC for the testing of new antibacterial agents.

Methods

Using Clinical and Laboratory Standards Institute (CLSI) methodology (CLSI 2015; CLSI 2016), minimal inhibitory concentration (MIC) values for antibiotics were determined for isolates in the FDA-CDC Antimicrobial Resistance Isolate Bank panels: 1) *Enterobacteriaceae* Carbapenem Breakpoint Panel ("Breakpoint"), 2) Gram-Negative Carbapenemase Detection Panel ("Detection") and 3) *Enterobacteriaceae* Carbapenemase Diversity Panel ("Diversity"). The Breakpoint panel contained 31 *Enterobacteriaceae* isolates, of which 7 were meropenem-resistant, including 5 bla_{KPC} isolates. The Detection panel was comprised of 80 isolates total; a 54 *Enterobacteriaceae* subset contained carbapenemase genes bla_{IMP} (n=2), bla_{KPC} (n=9), bla_{NDM} (n=10), bla_{OXA} (n=5), bla_{SME} (n=2), bla_{VIM} (n=3). The Diversity panel contained 53 *Enterobacteriaceae* with carbapenemase genes bla_{IMI} (n=2), bla_{IMP} (n=1), bla_{KPC} (n=18), bla_{NDM} (n=20), bla_{OXA} (n=5), bla_{SME} (n=6), and bla_{VIM} (n=2). There were 14 *A. baumannii* isolates across all panels, of which 4 isolates had bla_{NDM} and 8 had bla_{OXA} genes.

Beta-lactam antibiotic resistance classifications are listed as reported by the CDC. Isolates with tetracycline resistance were screened for the presence of tetracycline-specific resistance genes by PCR using published methodology (Grossman et al. 2012; Sutcliffe et al. 2013). Further, isolates of *Klebsiella pneumoniae* with elevated tigecycline and/or tetracycline-class antibiotic MIC values were screened for sequence variations in genes known to be associated with tigecycline non-susceptibility, namely the regulatory genes *ramR, acrR,* and *oqxR*, and *rpsJ*, which encodes ribosomal protein S10 (Bratu et al. 2009; Hentschke et al. 2010; Veleba and Schneiders 2012; Sheng et al. 2014; Beabout et al. 2015; Bialek-Davenet et al. 2015; Ahn et al. 2016).

Results

Table 1. Activity of Eravacycline and Comparators Against Gram-Negative Isolates from the CDC Antimicrobial Resistance Bank

	Panel				
Antibiotic	Carbapenem Breakpoint (N = 31) $MIC_{50/90}$ (Range)	Carbapenemase Detection (excluding *P.aeruginosa*) $MIC_{50/90}$ (Range)	Carbapenemase Diversity (N = 53) $MIC_{50/90}$ (Range)	All *Enterobacteriaceae* (N = 138) $MIC_{50/90}$ (Range)	All *A. baumannii* (N = 14) $MIC_{50/90}$ (Range)
Eravacycline	0.25/1 (0.031-2)	0.25/2 (0.031-4)	0.5/1 (0.031-8)	0.25/2 (0.031-8)	0.25/1 (0.031-2)
Tigecycline	0.25/1 (0.031-4)	0.5/4 (0.063-8)	0.5/2 (0.063-8)	0.5/2 (0.063-8)	1/4 (0.25-4)
Minocycline	8/32 (0.5->32)	4/>32 (≤0.016->32)	4/>32 (0.5->32)	8/>32 (0.5->32)	1/16 (≤0.016-16)
Tetracycline	8/>32 (0.5->32)	16/>32 (2->32)	16/>32 (0.5->32)	16/>32 (0.5->32)	>32 (2->32)
Meropenem	0.063/32 (≤0.016->32)	8/>32 (≤0.016->32)	>32 (1->32)	8/>32 (≤0.016->32)	>32 (2->32)
Ceftazidime	1/>32 (0.125->32)	>32 (0.25->32)	>32 (0.063->32)	>32 (0.063->32)	>32 (16->32)
Cefotaxime	0.5/>32 (0.031->32)	>32 (0.063->32)	>32 (0.125->32)	>32 (0.031->32)	>32 (>32)
Cefepime	0.5/>32 (0.063->32)	>32 (0.125->32)	>32 (0.125->32)	>32 (0.063->32)	>32 (8->32)
Colistin	0.125/1 (0.063->32)	0.25/>32 (0.063->32)	0.25/>32 (0.125->32)	0.25/>32 (0.063->32)	0.25/1 (0.063-1)
Gentamicin	1/>32 (0.25->32)	>32 (0.125->32)	16/>32 (0.25->32)	8/>32 (0.125->32)	>32 (8->32)
Levofloxacin	1/>32 (0.031->32)	16/>32 (0.031->32)	32/>32 (0.031->32)	16/>32 (0.031->32)	8/32 (4->32)
Piperacillin/ Tazobactam	16/4 />128/4 (1/4->128/4)	>128/4 (2/4->128/4)	>128/4 (1/4->128/4)	>128/4 (1/4->128/4)	>128/4 (128/4->128/4)
Aztreonam	4/>32 (≤0.016->32)	>32 (≤0.016->32)	>32 (≤0.016->32)	>32 (≤0.016->32)	>32 (32->32)
Trimethoprim/ Sulfamethoxazole	0.5/9.5 / >8/152 (0.063/1.19->8/152)	>8/152 (0.063/1.19->8/152)	>8/152 (0.125/2.38->8/152)	>8/152 (0.063/1.88->8/152)	>8/152 (8/152->8/152)

All values in μg/ml

$MIC_{50/90}$ and Range values for eravacycline and comparators against CDC Antimicrobial Resistance Bank panels. A single value is given when the MIC_{50} and MIC_{90} are equivalent. The Carbapenemase Detection panel is broken out into two columns showing values for all isolates, excluding *P. aeruginosa*, and all *Enterobacteriaceae* isolates. Data in "All *Enterobacteriaceae*" and "All *A. baumannii*" columns includes all isolates of the given classifications across the Breakpoint, Detection, and Diversity panels.

The eravacycline $MIC_{50/90}$ values and ranges against the full Breakpoint, Detection, and Diversity panels as well as subsets thereof are shown in Table 1. Overall, eravacycline maintained potency against isolates of *Enterobacteriaceae* and *A. baumannii*. The $MIC_{50/90}$ values for eravacycline against the Breakpoint and Diversity panels were 0.25/1 μg/ml and 0.5/1 μg/ml, the later a two-fold improvement over tigecycline. Against the Detection panel eravacycline maintained an $MIC_{50/90}$ of 0.5/2 μg/ml against isolates of *Enterobacteriaceae* alone, and 0.25/2 μg/ml against the total pool of *Enterobacteriaceae* and *A. baumannii* isolates, however, the eravacycline MIC values against the *P. aeruginosa* isolates (excluded from table, n=12) ranged from 2 to >32 μg/ml. Against the sum total of 138 *Enterobacteriaceae* and 14 *A. baumannii* eravacycline maintained an $MIC_{50/90}$ of 0.25/2 and 0.25/1 μg/ml, respectively.

Table 2. Activity of Eravacycline and Comparators against *Enterobacteriaceae* with Defined Resistance Mechanisms

		$MIC_{50/90}$ (Range)			
	N=	Eravacycline	Tigecycline	Tetracycline	Meropenem
bla_{NDM}	30	0.5/2 (0.031-4)	1/4 (0.063-4)	>32 (0.5->32)	>32 (4->32)
bla_{KPC}	32	0.25/2 (0.031-8)	0.5/2 (0.063-8)	8/>32 (0.5->32)	8/>32 (0.25->32)
bla_{CTXM}	8	n/a (0.125-8)	n/a (0.125-8)	n/a (4->32)	n/a (0.125->32)
bla_{OXA}	11	0.5/1 (0.031-1)	0.5/2 (0.25-2)	>32 (2->32)	2/16 (0.5->32)
bla_{TEM}	6	n/a (0.063-0.5)	n/a (0.125-1)	n/a (4->32)	n/a (0.125-8)
bla_{SHV}	4	n/a (0.125-2)	n/a (0.25-2)	n/a (4->32)	n/a (0.25-8)
bla_{OXA}	1	n/a (0.5)	n/a (0.5)	n/a (>32)	n/a (16)
bla_{CMY}	3	n/a (0.031-0.25)	n/a (0.063-0.25)	n/a (4->32)	n/a (0.031-0.5)
cAmpC	5	n/a (0.125-2)	n/a (0.25-2)	n/a (4->32)	n/a (≤0.016-0.5)
Porin-Loss	5	n/a (0.125-0.5)	n/a (0.25-0.5)	n/a (4->32)	n/a (0.5-32)
bla_{VIM}	5	n/a (0.063-4)	n/a (0.25-8)	n/a (2->32)	n/a (4->32)
bla_{IMI}	2	n/a (0.125-0.125)	n/a (0.125)	n/a (4)	n/a (32->32)
bla_{IMP}	3	n/a (0.125-0.25)	n/a (0.25-0.5)	n/a (2)	n/a (4-8)
bla_{SME}	8	n/a (0.5-1)	n/a (0.5-1)	n/a (8-32)	n/a (32->32)
ramR, acrR, oqxR variant	17	1/4 (0.125-4)	2/4 (0.25-8)	16/>32 (4->32)	>32 (0.031->32)
tet Gene	49	0.5/2 (0.031-8)	0.5/4 (0.063-8)	>32 (8->32)	8/>32 (≤0.016->32)

All values in μg/ml

$MIC_{50/90}$ and Range values for eravacycline and comparators against *Enterobacteriaceae* isolates with defined resistance characteristics. A single value is given when the MIC_{50} and MIC_{90} or MIC maximum and minimum values are equivalent. n/a: $MIC_{50/90}$ values are not calculated for populations of less than 10 isolates. Isolates are compiled from Breakpoint, Detection, and Diversity panels. Carbapenemase genes are presented as listed by the CDC Antimicrobial Resistance Bank.

The presence of various beta-lactamase resistance mechanisms did not impact the activity of eravacycline against isolates of *Enterobacteriaceae*. Across all *Enterobacteriaceae* carrying the bla_{NDM} (n=30) and bla_{KPC} (n=32) genes, two major carbapenem resistance mechanisms, eravacycline remained potent, maintaining $MIC_{50/90}$ values of 0.5/2 μg/ml and 0.25/2 μg/ml, respectively. Similarly, against a total of 49 isolates carrying one or more of the tetracycline-specific efflux pump genes *tet*(A), *tet*(B), *tet*(C), or *tet*(D), eravacycline showed an $MIC_{50/90}$ of 0.5/2 μg/ml, while in isolates of *K. pneumoniae* carrying sequence variants of genes known to be associated with reduced susceptibility to tigecycline (n=17), eravacycline showed a modestly reduced susceptibility with an $MIC_{50/90}$ of 1/4 μg/ml.

Table 3. Activity of Eravacycline and Comparators against *A. baumannii* with Defined Resistance Mechanisms

		$MIC_{50/90}$ (Range)			
	N=	Eravacycline	Tigecycline	Tetracycline	Meropenem
bla_{NDM}	4	n/a (0.031-0.25)	n/a (0.125-1)	n/a (2->32)	n/a (>32)
bla_{OXA}	8	n/a (0.031-1)	n/a (0.25-4)	n/a (4->32)	n/a (2->32)
tet Gene	8	n/a (0.031-1)	n/a (0.125-4)	n/a (16->32)	n/a (4->32)

All values in μg/ml

$MIC_{50/90}$ and Range values for eravacycline and comparators against *A. baumannii* isolates with defined resistance characteristics. A single value is given when the MIC_{50} and MIC_{90} or MIC maximum and minimum values are equivalent. n/a: $MIC_{50/90}$ values are not calculated for populations of less than 10 isolates. Isolates are compiled from Breakpoint, Detection, and Diversity panels. Carbapenemase genes are presented as listed by the CDC Antimicrobial Resistance Bank.

Beta-lactam resistance mechanisms did not impact the activity of eravacycline against isolates of *A. baumannii* with MIC values ranging from 0.031 to 1 μg/ml for isolates carrying bla_{NDM} (n=4), bla_{OXA} (n=8), and/or tetracycline-specific resistance genes (n=8), maintaining four-fold potency over tigecycline MIC ranges in each set of isolates.

Conclusions

Eravacycline maintained potency against carbapenem-resistant *Enterobacteriaceae* and *A. baumannii* isolates in the CDC Antimicrobial Resistance Bank, including those containing carbapenemase genes prevalent in contemporary clinical isolates.

References

1. Ahn C, Yoon SS, Yong TS, Jeong SH, Lee K. 2016. The Resistance Mechanism and Clonal Distribution of Tigecycline-Nonsusceptible Klebsiella pneumoniae Isolates in Korea. *Yonsei Medical Journal* 57: 641-646.
2. Beabout K, Hammerstrom TG, Perez AM, Magalhaes BF, Prater AG, Clements TP, Arias CA, Saxer G, Shamoo Y. 2015. The ribosomal S10 protein is a general target for decreased tigecycline susceptibility. *Antimicrob Agents Chemother* 59: 5561-5566.
3. Bialek-Davenet S, Lavigne JP, Guyot K, Mayer N, Tournebize R, Brisse S, Leflon-Guibout V, Nicolas-Chanoine MH. 2015. Differential contribution of AcrAB and OqxAB efflux pumps to multidrug resistance and virulence in Klebsiella pneumoniae. *J Antimicrob Chemother* 70: 81-88.
4. Bratu S, Landman D, George A, Salvani J, Quale J. 2009. Correlation of the expression of acrB and the regulatory genes marA, soxS and ramA with antimicrobial resistance in clinical isolates of Klebsiella pneumoniae endemic to New York City. *J Antimicrob Chemother* 64: 278-283.
5. CLSI. 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically M07-A10. Clinical and Laboratory Standards Institute.
6. CLSI. 2016. Performance standards for antimicrobial susceptibility testing, 26th Edition, CLSI Supplement M100S. Clinical and Laboratory Standards Institute.
7. Grossman TH, Starosta AL, Fyfe C, O'Brien W, Rothstein DM, Mikolajka A, Wilson DN, Sutcliffe JA. 2012. Target- and Resistance-Based Mechanistic Studies with TP-434, a Novel Fluorocycline Antibiotic. *Antimicrobial Agents and Chemotherapy* 56: 2559-2564.
8. Hentschke M, Wolters M, Sobottka I, Rohde H, Aepfelbacher M. 2010. ramR mutations in clinical isolates of Klebsiella pneumoniae with reduced susceptibility to tigecycline. *Antimicrob Agents Chemother* 54: 2720-2723.
9. Sheng Z-K, Hu F, Wang W, Guo Q, Chen Z, Xu X, Zhu D, Wang M. 2014. Mechanisms of Tigecycline Resistance among Klebsiella pneumoniae Clinical Isolates. *Antimicrobial Agents and Chemotherapy* 58: 6982-6985.
10. Sutcliffe JA, O'Brien W, Fyfe C, Grossman TH. 2013. Antibacterial Activity of Eravacycline (TP-434), a Novel Fluorocycline, against Hospital and Community Pathogens. *Antimicrobial Agents and Chemotherapy* 57: 5548-5558.
11. Veleba M, Schneiders T. 2012. Tigecycline resistance can occur independently of the ramA gene in Klebsiella pneumoniae. *Antimicrob Agents Chemother* 56: 4466-4467.