Presentation: 451
Saturday

Analysis of Baseline Pathogens and Clinical Efficacy in IGNITE1, a Phase 3 Study to Evaluate the Efficacy and Safety of Eravacycline (ERV) versus Ertapenem (ETP) in Complicated Intra-Abdominal (cIAI) Infections

J. Sutcliffe[1], A. Marsh[1], S. Izmailyan[1], L. Tsai[1], M. Castanheira[2], J. Solomkin[3], P. Horn[1]
[1]Tetraphase Pharmaceuticals, Watertown MA; [2]JMI Laboratories, North Liberty IA; [3]Univ. of Cincinnati Coll. of Med., Cincinnati, OH

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 being developed for the treatment of serious infections, including those caused by MDR pathogens. ERV was evaluated in a phase 3, randomized, double blind, multicenter study where patients with cIAI received either ERV (1.0 mg/kg IV BID) or ETP (1g IV QD). ERV demonstrated non-inferiority to ertapenem in the primary analysis. This analysis evaluated patient outcomes based on the resistant phenotypes and genotypes present in baseline Gram-negative pathogens.

Methods: Baseline blood and cIAI specimens were obtained from all randomized patients. Susceptibility to study antibiotics and relevant comparators was determined by CLSI broth microdilution methods. Isolates of the same bacterial species from a single subject were evaluated by pulsed-field gel electrophoresis (PFGE) to assess genetic relatedness. Gram-negative aerobes resistant to any 3rd/4th generation cephalosporin and/or carbapenem were screened using published PCR conditions for narrow- and extended-spectrum β-lactamases (ESBL) and carbapenemases. Overexpression of chromosomal AmpC β-lactamases (↑AmpC) was evaluated.

Results: 541 patients were randomized; baseline isolates were cultured from 446 patients. Following PFGE testing of 708 isolates, 1353 unique isolates were identified among the 446 patients. 151 Gram-negative isolates were tested for β-lactamase resistance mechanisms. CTX-M enzymes were the most common ESBLs among Enterobacteriaceae. KPC, IMP, VIM and OXA carbapenemases were also detected.

IGNITE 1: Favorable Outcomes[1] (Clinical Cure at TOC in the micro-ITT Population) for Patients with Gram-Negative Pathogens		
Pathogen	ERV	ETP
All baseline pathogens	191/220 (86.8)	198/226 (87.6)
Gram-negative pathogens	155/182 (85.2)	162/186 (87.1)
CEPH-R[2]	25/27 (92.6)	24/28 (85.7)
Confirmed ESBL	22/24 (91.7)	16/19 (84.2)
Confirmed ↑AmpC	8/8 (100)	7/8 (87.5)
Carbapenem-resistant	6/6 (100)	8/9 (88.9)
Confirmed carbapenemase	4/4 (100)	4/5 (80.0)
MDR[3]	18/20 (90.0)	15/16 (93.8)

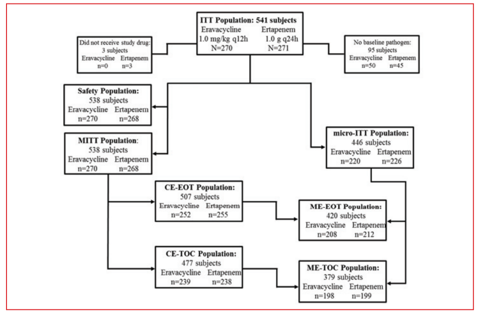
[1] Results are presented as n/N (%), where n=subjects with favorable outcomes, N=total subjects in the category, and % = n/N*100; [2] CEPH-R=3rd/4th generation cephalosporin-resistant according to 2015 CLSI M100 guidance; [3] Multidrug-resistance according to modified ESCMID criteria[6]

Conclusions: ERV was efficacious in patients with cIAI including those infected with difficult-to-treat Gram-negative pathogens.

Introduction

- Eravacycline is a novel, fully-synthetic fluorocycline antibiotic of the tetracycline class with potent in vitro activity against antibiotic-resistant bacteria[3,4], including those identified as urgent or serious threats by the CDC[7].
- IGNITE1 entitled "A Phase 3, Randomized, Double-Blind, Double-Dummy, Multicenter, Prospective Study to Assess the Efficacy and Safety of Eravacycline Compared with Ertapenem in Complicated Intra-abdominal Infections" was designed to assess the efficacy, safety, and tolerability of eravacycline compared with ertapenem in adults with cIAIs and to assess the PK of eravacycline[5].
- The primary efficacy analysis was based on the micro-ITT population and examined clinical response at the Test of Cure (TOC) visit, 25-31 days after the initiation of study drug treatment.
- The difference in clinical cure rates between treatment groups was determined along with the 95% confidence interval (CI). The non-inferiority margin for this analysis was 10%.
- Qualified subjects enrolled in the study were randomized to 1 of 2 treatment groups, eravacycline 1.0 mg/kg q12h, or ertapenem 1 g q24h IV, in a 1:1 ratio. Randomization was stratified based on the primary site of infection (complicated appendicitis versus all other diagnoses).
- Duration of treatment with study drug (4 to 14 days) was based upon clinical response and was continued until resolution and documentation of signs and symptoms of infection.

IGNITE1 Analysis Populations



Abbreviations: CE = all subjects who met the definition for the ITT population and had no major protocol deviations (as defined in the SAP); cIAI = complicated intra-abdominal infection; EOT = End of Treatment Visit; ITT = all randomized subjects; ME = all subjects who met the definition for the micro-ITT population and had no major protocol deviations (as defined in the SAP); micro-ITT = all randomized subjects who had baseline bacterial pathogens that caused cIAI and against at least one of which the investigational drug has in vitro antibacterial activity; MITT = modified intent to-treat, all treated; N = number of subjects in the primary analysis population; n = number of subjects in the subgroup or treatment group; q12h = every 12 hours; q24h = every 24 hours; Safety = all subjects who received at least 1 dose of study drug; TOC = Test of Cure Visit.

- Approximately 27% of randomized subjects had a primary disease diagnosis of complicated appendicitis, and the remaining 73% had a primary disease diagnosis of "Other cIAI."
- Analyses of baseline pathogens revealed a similar distribution of monomicrobial and polymicrobial infections. In subjects with monomicrobial infections, Gram-negative aerobes were isolated over twice as frequently as Gram-positive aerobes. Anaerobic monomicrobial infections were relatively few.
- In subjects with polymicrobial infections, the most common combination was Gram-negative aerobes with both Gram-positive aerobes and anaerobes (49 [22.3%] and 50 [22.1%] subjects in the eravacycline and ertapenem groups, respectively).

Materials and Methods

Antibacterial agents

- Eravacycline (Tetraphase Pharmaceuticals) and ertapenem (Eurofins Medinet)

Bacterial strain collection and in vitro susceptibility analysis

- Isolates were collected with kits and materials supplied by the central laboratories at Eurofins Medinet.
- Isolates were initially speciated by the local/regional lab and speciation was confirmed by the central laboratory.
- Minimal inhibitory concentrations (MICs) were determined by broth microdilution according to Clinical Laboratory Standards Institute (CLSI)[1]. CLSI interpretive criteria from 2015 were applied[2].

PFGE inclusion criteria

- For subjects that had multiple isolates of the same bacterial species, regardless of study visit, those isolates were analyzed by PFGE for clonal relatedness.
- Genomic DNA was prepared in agarose blocks and digested with restriction endonucleases (New England, Beverly, Massachusetts, USA). Electrophoresis was performed on the CHEF-DR III (BioRad, Richmond, California), with the following standard settings 0.5 x TBE, 1 % agarose, 13°C and 200V; running conditions were genus/species-specific as described elsewhere.
- Gel pattern analysis was performed using the GelCompar II software (Applied Math, Kortrijk, Belgium).

β-Lactamase characterization

- Enterobacteriaceae
  - Enterobacteriaceae with unique PFGE patterns that met the susceptibility screening criteria for ESBL according to CLSI guidelines[2] were screened for ESBLs, inhibitor-resistant ESBLs and plasmidic AmpC-encoding genes.
  - Enterobacteriaceae isolates exhibiting non-susceptibility to imipenem (MIC ≥2 µg/mL) were also screened for carbapenemase-encoding genes.
- Non-fermentative Gram-negative organisms
  - *Pseudomonas* spp. and *Acinetobacter* spp. and other non-fermentative organisms with unique PFGE patterns and ceftazidime MIC values ≥16 µg/mL were screened for ESBL-encoding genes and the transcription level of chromosomally-encoded *ampC*.
  - Microarray-based (Check-MDR CT101; Wageningen, The Netherlands) and/or PCR reactions targeting common β-lactamase-encoding genes were performed using purified total genomic DNA extracts from all unique isolates. All amplicons generated were sequenced on both strands for confirmation of PCR products and determination of allelic variants. Nucleotide and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI, USA). Amino acid sequences were compared with those available through the internet using BLAST (http://www.ncbi.nlm.nih.gov/blast/).
- Strains with imipenem MIC values ≥8 µg/mL were screened for carbapenemase genes. OprD levels were determined by Western Blot for *P. aeruginosa* isolates displaying imipenem MIC values of ≥8 µg/mL. OprD loss/decrease for *P. aeruginosa* isolates was assessed by sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and by Western blot analysis with an anti-OprD antibody. Results were compared to those of *P. aeruginosa* PAO1 control strain.
- Expression of chromosomally-encoded *ampC* for *P. aeruginosa* and selected Enterobacteriaceae species was quantified using total mRNA from log phase bacterial cultures and was performed by real-time PCR in triplicate reactions that were normalized to an endogenous reference gene. The *ampC* expression from clinical isolates was compared to those from reference strains and transcription levels was considered significantly higher if at least a 10-fold difference was observed.

Results

Microbiological and Clinical Outcomes for micro-ITT Subjects with Gram-Negative Bacilli Pathogens Containing One or More β-lactamases

Treatment group	Organisms	MIC (µg/ml)						β-lactamase																																Per pathogen micro outcome TOC	Clinical outcome TOC
		ERAVACYCLINE	CEFOTAXIME	CEFEPIME	CEFTAZIDIME	ertapenem	IMIPENEM	GES-11	GES-17	CTX-M-1-like	CTX-M-14	CTX-M-15-like	CTX-M-2	CTX-M-24	CTX-M-27	CTX-M-3-like	CTX-M-3	CTX-M-55/79	OXA-1/30	OXA-10	OXA-72-like	PER-1	SFO-1-like	SHV-1-like	SHV-1	SHV-11	SHV-12	SHV-121	SHV-30	SHV-31	TEM-1	TEM-30	AmpC_Overexpression	IMP-1	KPC-2	OXA-23	OXA-48	OXA-72-like	VIM-2		
ERV	*ACHROMOBACTER DENITRIFICANS*	8	>8	>128	4	0.02	0.25																																	PE	C
ERV	*ACINETOBACTER BAUMANNII* COMPLEX	0.12	>8	8	>16	2	0.25																									X								PE	C
ERV	*ACINETOBACTER BAUMANNII* COMPLEX	0.25	>8	>128	>16	>16	8	X																																PE	C
ERV	*ACINETOBACTER BAUMANNII* COMPLEX	0.25	>8	>128	>16	>16	2	X																																PE	C
ERV	*ACINETOBACTER BAUMANNII* COMPLEX	0.25	>8	128	>16	4	1															X											X							PE	C
ERV	*ACINETOBACTER BAUMANNII* COMPLEX	0.25	>8	>128	>16	>16	4	X																									X							PE	C
ERV	*ACINETOBACTER BAUMANNII* COMPLEX	0.25	>8	>128	>16	>16	4	X																									X							PE	C
ERV	*ACINETOBACTER BAUMANNII* COMPLEX	0.5	>8	32	>16	>16	>8																										X					X		PE	C
ERV	*ACINETOBACTER BAUMANNII* COMPLEX	0.5	>8	4	>16	2	0.25																																	PE	C
[illegible]	[illegible]																																								

Favorable Outcomes (Clinical Cure at TOC in the micro-ITT Population) for Subjects with Gram-Negative Bacilli Pathogens

Pathogen	ERV	ETP
All baseline pathogens	191/220 (86.8)	198/226 (87.6)
Gram-negative pathogens	155/182 (85.2)	162/186 (87.1)
CEPH-R[2]	25/27 (92.6)	24/27 (88.9)
Confirmed ESBL[3]	22/24 (91.7)	16/18 (88.9)
Confirmed ↑AmpC	9/9 (100)	6/7 (85.7)
CP-R[4]	6/6 (100)	8/9 (88.9)
Confirmed carbapenemase[5]	4/4 (100)	4/5 (80.0)
KPC-2	1/1 (100)	-
IMP-1	1/1 (100)	-
VIM-2	-	1/1 (100)
OXA-23	1/1 (100)	1/1 (100)
OXA-48	-	0/1 (0)
OXA-72-like	1/1 (100)	2/2 (100)
MDR[6]	18/20 (90.0)	16/17 (94.1)
Enterobacteriaceae	142/168 (84.5)	147/171 (86.0)
CRE	1/1 (100.0)	4/5 (80.0)
Confirmed CRE	1/1 (100)	0/1 (0.0)
CEPH-R Enterobacteriaceae	18/20 (90.0)	18/22 (81.8)
Confirmed ESBL	19/21 (90.5)	15/18 (83.3)
MDR Enterobacteriaceae	14/16 (87.5)	11/12 (91.7)
Gram-negative aerobes other than Enterobacteriaceae	37/44 (84.1)	32/34 (94.1)
Pseudomonas aeruginosa	15/18 (83.3)	18/20 (90)
Stenotrophomonas maltophilia	1/3 (33.3)	1/1 (100)
Acinetobacter species	8/8 (100)	6/6 (100)
CEPH-R *Acinetobacter* species	8/8 (100)	5/5 (100)
A. baumannii complex	8/8 (100)	5/5 (100)
CRAB	3/3 (100)	4/4 (100)
Confirmed CRAB	2/2 (100)	4/4 (100)
Confirmed ESBL	5/5 (100)	1/1 (100)
MDR A. baumannii	7/7 (100)	4/4 (100)
Other non-Enterobacteriaceae[7]	3/3 (100)	-

NOTE: Results are presented as n/N (%), where n=subjects with favorable outcomes, N=total subjects in the category, and % = n/N*100; Clinical response is based on the Surgical Adjudication Committee assessment; [2]CEPH -R=3rd/4th generation cephalosporin-resistant according to 2015 CLSI breakpoints; [3] one or more: GES-11, CTX-M-14, CTX-M-15-like, CTX-M-1-like, CTX-M-3-like, CTX-M-55/79, PER-1, SFO-1-like, SHV-11, SHV-12, SHV-30;[4] CP-R=carbapenem resistant according to 2015 CLSI breakpoints;[5] IMP-1, KPC-2, OXA-23, OXA-48, OXA-72-like, or VIM-2;[6] MDR=multidrug resistant according to modified ESCMID criteria (Magiorakos, et al., 2011[6]); [7]*Achromobacter denitrificans* (n=1), *Chryseobacterium indologenes* (n=1), *Ochrobactrum anthropic* (n=1)

Favorable Outcomes (Clinical Cure at TOC in the micro-ITT Population) for Subjects with Gram-Positive Aerobic and Gram-Negative Anaerobic Pathogens

Pathogen	ERV	ETP
Gram-positive pathogens	100/117 (86.3)	105/120 (87.5)
Streptococcus spp.	58/65 (89.2)	54/64 (84.4)
Streptococcus anginosus	25/29 (86.2)	9/12 (75.0)
Streptococcus constellatus	13/15 (86.7)	10/14 (71.4)
Streptococcus mitis	6/7 (85.7)	16/17 (94.1)
Enterococcus faecalis	16/23 (69.6)	21/26 (80.8)
Enterococcus faecium	13/16 (81.3)	26/30 (86.7)
Anaerobes	89/106 (83.0)	94/107 (87.9)
Bacteroides spp.	72/87 (82.8)	74/84 (88.1)
Bacteroides fragilis	39/44 (88.6)	38/42 (90.5)
Bacteroides thetaiotaomicron	23/26 (88.5)	17/20 (85.0)
Bacteroides ovatus	13/19 (68.4)	15/17 (88.2)
Bacteroides vulgatus	10/12 (83.3)	14/17 (82.4)

Abbreviations: micro-ITT = all randomized subjects who had baseline bacterial pathogens that caused cIAI and against at least one of investigational drug had in vitro antibacterial activity; N = number of subjects with the specified baseline pathogen; n = number of subjects within a specific category; TOC = Test of Cure visit.
Notes: Percentages were calculated as 100 × (n/N). Clinical response is based on the Surgical Adjudication Committee assessment.
Subjects with the same pathogen from more than 1 specimen were counted only once for that pathogen. Subjects were counted only once in the overall tabulation of Gram-negative aerobes, Gram-negative anaerobes, and Gram positive aerobes. Subjects are counted only once for the overall tabulation, *Bacteroides* spp., and *Streptococcus* spp.

Summary

- Eravacycline is a novel, fully-synthetic fluorocycline antibiotic of the tetracycline class being developed for the treatment of complicated urinary tract infections, including pyelonephritis, and complicated intra-abdominal infections.
- Susceptibility to eravacycline in this phase 3 cIAI trial did not appear to be influenced by the presence of one or more β-lactamase genes, including those encoding extended-spectrum β-lactamases and/or class A – D carbapenemases.
- Eravacycline MIC values ranged from 0.06–1 µg/mL for *Acinetobacter* spp., 0.25-1 µg/mL for *Citrobacter freundii* and *Enterobacter cloacae*, 0.03-0.5 µg/mL for *Escherichia coli*, 0.12-2 µg/mL for *K. pneumoniae*, 1-2 µg/mL for *Morganella morganii* and *Proteus mirabilis*, 2-16 µg/mL for *Pseudomonas aeruginosa*, 1-2 µg/mL for *Serratia marcescens*.
- In the IGNITE1 study, eravacycline demonstrated high clinical cure rates against Gram-negative pathogens that were carbapenem-resistant, 3rd/4th generation cephalosporin-resistant, or multidrug-resistant.

References

1. CLSI. M07-A9: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - Ninth Edition; (2013).
2. CLSI. M100-S25: Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Information Supplement; (2015).
3. Abdallah, M. et al.. Antimicrob. Agents Chemother. 59, 1802 (2015).
4. Sutcliffe, J. A., et al. Antimicrob. Agents Chemother. 57, 5548 (2013).
5. Solomkin J, et al., abstr. LBOS0239d, 25th ECCMID, (2015).
6. Magiorakos, A. P., et al. Clin Microbiol Infect. 18, 268 (2012). doi: 10.1111/j.1469-0691.2011.03570.x. Epub 2011 Jul 27.
7. CDC. Antibiotic resistance threats in the United States, 2013.

Presented at ASM Microbe 2016, June 16 - June 20, Boston, Massachusetts, USA