Session 062
Friday-603

The Novel β-lactamase Inhibitor ETX1317 Effectively Restores the Activity of Cefpodoxime Against Extended Spectrum β-lactamase (ESBL)- and Carbapenemase-Expressing *Enterobacteriaceae* Isolated From Recent Urinary Tract Infections

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Abstract

Background

The treatment of urinary tract infections (UTIs) has been complicated by the emergence of multi-drug resistant, β-lactamase (BLA)-expressing pathogens. ETX0282 is an oral prodrug which is hydrolyzed *in vivo* to release ETX1317, a novel diazabicyclooctenone β-lactamase inhibitor (BLI) active against serine BLAs. ETX1317 has intrinsic antibacterial activity against some *Enterobacteriaceae* but this *in vitro* activity does not translate to preclinical *in vivo* efficacy when dosed alone. ETX0282 is currently under investigation in combination with cefpodoxime proxetil (CPDP), a clinically approved antibiotic which is hydrolyzed *in vivo* to release cefpodoxime (CPD). We sought to determine the optimal susceptibility testing paradigm for CPD-ETX1317, then tested the combination against a collection of geographically diverse BLA-producing UTI isolates.

Methods

In vitro bacterial susceptibility to CPD -/+ ETX1317 at several fixed concentrations, or CPD- ETX1317 in different ratios was determined against 59 *Enterobacteriaceae* clinical isolates selected based on their BLA gene content and their range of susceptibilities to CPD and ETX1317 alone. CPD-ETX1317 was then tested in a fixed 1:2 ratio against 910 BLA-enriched *Enterobacteriaceae* (including *Escherichia coli*, *Klebsiella, Citrobacter*, *Proteus* and *Enterobacter* spp.) collected between 2013-2015 from geographically diverse medical centers around the world. Susceptibility testing was performed according to CLSI guidelines.

Results

Against some isolates, titrations of CPD with fixed concentrations of ETX1317 resulted in MIC values below the limit of detection, presumably due to the intrinsic antibacterial activity of ETX1317. Based on scatter plots, MIC_{50} and MIC_{90} values, maximal potency of the CPD-ETX1317 combination was observed at a 1:2 ratio. The MIC_{90} of CPD against 910 BLA-enriched *Enterobacteriaceae* improved from >32 mg/L to 0.5 mg/L in the presence of ETX1317 at a 1:2 ratio. Effective restoration of CPD activity was observed across all bacterial species and serine BLA classes tested, including isolates expressing ESBL, AmpC-overexpressing, KPC and OXA-48-like enzymes.

Conclusions

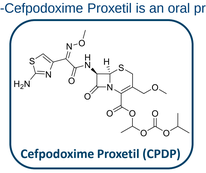
CPD-ETX1317 in a 1:2 fixed ratio is the testing paradigm most likely to reflect the contribution of both partners in a β-lactam-BLI combination, and therefore may represent the optimal correlation to *in vivo* efficacy. ETX1317 potently restores the activity of CPD against ESBL-producing and/or carbapenem-resistant *Enterobacteriaceae* using this testing paradigm.

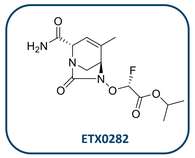
Introduction

95% of UTIs are caused by *Enterobacteriaceae*[1]. Emergence of multi-drug resistant (MDR) bacteria, including fluoroquinolone-resistant, AmpC β-lactamase-, ESBL- and carbapenemase-producing strains of *Enterobacteriaceae*, has complicated treatment of patients with these infections. Resistance to existing oral therapies for UTI is forcing physicians to unnecessarily admit patients and administer lengthy IV treatment resulting in excessive healthcare expenses. Many physicians identify the lack of a potent, oral Gram-negative agent as one of the field's biggest unmet needs[2]. In response to this challenge, Entasis Therapeutics is developing an oral Gram-negative drug targeting complicated UTI (cUTI) infections, including those caused by carbapenem-resistant *Enterobacteriaceae* (CRE). The agent is a combination of cefpodoxime-proxetil plus the diazabicyclooctenone prodrug, ETX0282 which is metabolized *in vivo* to cefpodoxime (CPD) and ETX1317. The intended use for this product is as first line treatment for cystitis and pyelonephritis in outpatient settings or as oral step-down therapy in hospital settings, resulting in significant reduction of healthcare costs.

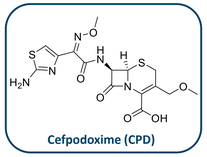
A Broad-spectrum Oral β-lactam/β-lactamase Inhibitor Combination

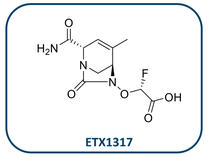
ETX0282-Cefpodoxime Proxetil is an oral prodrug combination





The oral combination is hydrolyzed *in vivo* to release the active moieties Cefpodoxime-ETX1317





ETX1317 is a β-lactamase inhibitor that inhibits class A, C and selected class D β-lactamases. ETX1317 also has intrinsic antibacterial activity against select species.

Development of the Cefpodoxime-ETX1317 Testing Paradigm

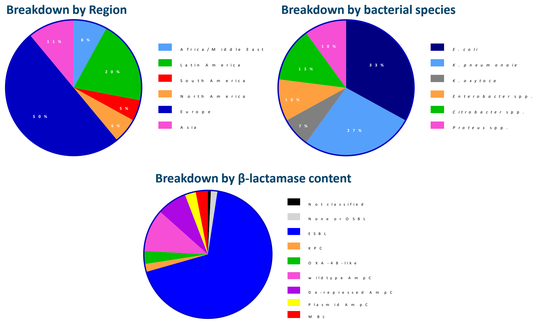
To determine the concentration or ratio of ETX1317 combined with CPD that results in optimum potency and an on-scale *in vitro* MIC testing paradigm, bacterial susceptibility to CPD in the presence of fixed concentrations of ETX1317 or CPD titrated in a fixed ratio with ETX1317 was measured. Each combination was tested against 59 *Enterobacteriaceae* clinical isolates. These isolates were chosen based on β-lactamase gene content and range of susceptibilities to cefpodoxime alone and ETX1317 alone.

	Number (cumulative %) of isolates inhibited at MIC (mg/L)												
Antimicrobial	**≤0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**>64**
Cefpodoxime alone			6	3	3	1	1	1	1	3	2	2	**36**
	0%	0%	10%	15%	20%	22%	24%	25%	27%	32%	36%	39%	**100%**
Cefpodoxime-ETX1317 (1:2)		14	21	15	1	**5**	2		1				
	0%	24%	59%	85%	86%	**95%**	98%	98%	100%	100%	100%	100%	100%
Cefpodoxime-ETX1317 (1:1)		4	14	20	11	3	**4**	2	1				
	0%	7%	31%	64%	83%	88%	**95%**	98%	100%	100%	100%	100%	100%
Cefpodoxime-ETX1317 (2:1)			9	10	13	17	**4**	3	2	1			
	0%	0%	15%	32%	54%	83%	**90%**	95%	98%	100%	100%	100%	100%
Cefpodoxime-ETX1317 (4:1)				4	6	9	20	13	**3**	1	2	1	
	0%	0%	0%	7%	17%	32%	66%	88%	**93%**	95%	98%	100%	100%
Cefpodoxime + 1 mg/L ETX1317	50	2			**1**	1	2				1	3	
	85%	88%	88%	88%	**90%**	92%	93%	93%	93%	93%	95%	100%	100%
Cefpodoxime + 2 mg/L ETX1317	**54**	1	1									3	
	92%	93%	95%	95%	95%	95%	95%	95%	95%	95%	95%	100%	100%
Cefpodoxime + 4 mg/L ETX1317	**58**											1	
	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	98%	100%	100%
ETX1317 alone				13	19	4		5	5	**8**	5		
	0%	0%	0%	22%	54%	61%	61%	69%	78%	**92%**	100%	100%	100%

- Due to the intrinsic activity of ETX1317, titrations of CPD with fixed concentrations of ETX1317 resulted in MIC values below the limit of detection.
- CPD-ETX1317 titrated in a 1:2 ratio is the optimal testing paradigm to demonstrate contribution of both partners, which translates to potent activity against a wide range of β-lactamase-expressing isolates.

Enterobacteriaceae Susceptibility: Study Design

Organisms:
All IHMA cUTI isolates were collected from geographically diverse medical centers located in North America, Latin America, South America, Europe, Middle East, Africa and the Asia-Pacific region during 2013, 2014 and 2015. Isolates were enriched for ESBL+ genotypes and consisted of approximately one-third *E. coli*, one-third *Klebsiella* spp., 10% each *Enterobacter*, *Citrobacter* and *Proteus* spp.



Methods:
Broth microdilution susceptibility testing was conducted according to CLSI guidelines[3] using cation-adjusted Mueller-Hinton broth. Where indicated, CPD-ETX1317 = MIC of cefpodoxime titrated with ETX1317 in a 1:2 ratio; CAZ-AVI = MIC of ceftazidime in the presence of 4 mg/L avibactam; PIP-TAZ = MIC of piperacillin in the presence of 4 mg/L tazobactam. Glucose-6-phosphate was added at 25 mg/L for fosfomycin (FOS) susceptibility testing.

Cumulative Activity vs. 910 ESBL-enriched *Enterobacteriaceae*

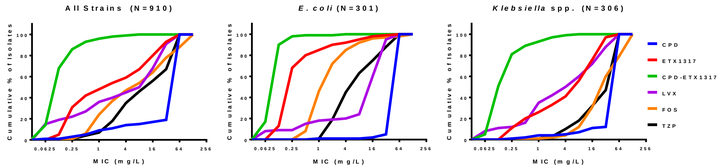
		Number (cumulative %) of isolates inhibited at MIC (mg/L)											
Antimicrobial	**%S***	**≤ 0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**>32**
CPD	11		5	6	14	19	34	20	27	17	18	18	**732**
			0.5%	0.12%	2.7%	4.8%	8.6%	10.8%	13.7%	15.6%	17.6%	19.5%	**100%**
ETX1317	NA	1	0	46	234	98	54	59	44	78	113	115	**68**
		0%	0%	5%	31%	42%	48%	54%	59%	67%	80%	93%	**100%**
CPD-ETX1317 (1:2)	98	6	117	499	164	**57**	30	18	13	3	2	1	
		0.7%	13.5%	68.4%	86.4%	**92.6%**	95.9%	97.9%	99.3%	99.7%	99.9%	100%	
FOS	88					5	53	160	122	82	66	97	120	**202**
						1%	6%	24%	37%	47%	54%	64%	78%	**100%**
LVX	40		140	35	29	46	77	39	40	48	163	**207**	86
			15%	19%	22%	27%	36%	40%	45%	50%	68%	**91%**	100%
TMX	34					278	12	19	16	**585 (≥ 8)**			
						31%	32%	34%	36%	**100%**			
TZP	56				8	26	30	95	161	99	92	101	**295**
					1%	4%	7%	18%	35%	46%	56%	67%	**100%**

CPD = cefpodoxime; FOS = fosfomycin; LVX = levofloxacin; TZP = piperacillin-tazobactam; NA = applicable; MIC_{90}s are highlighted with blue squares.
*based on 2018 CLSI breakpoint criteria[4]. CPD-ETX1317 breakpoint is based on the CLSI 2016 CPD breakpoint of ≤2 mg/L.

- CPD has a MIC_{90} of >32 mg/L, which is improved by >64-fold to to 0.5 mg/L in the presence of ETX1317.
- CPD-ETX1317 was the most potent oral agent tested against this set of 910 clinical isolates of *Enterobacteriaceae*.

Activity of Cefpodoxime-ETX1317 by Bacterial Species

Bacterial Species	N	Range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Number (%) of MBL+
E. coli	301	≤0.015 - 4	0.12	0.12	0 (0)
***Klebsiella* spp.**	306	0.06 - 16	0.12	1	4 (1.3)
***Citrobacter* spp.**	120	0.03 - 16	0.12	0.5	7 (5.8)
***Enterobacter* spp.**	90	0.06 - 4	0.25	1	12 (12.2)
***Proteus* spp.**	93	≤0.015 - 32	0.12	1	5 (5.3)
All isolates	910	≤0.015 - 32	0.12	0.5	28 (3.0)



Activity of Cefpodoxime-ETX1317 by β-lactamase Class

β-lactamase class	All	Not known or None	ESBL	KPC	OXA-48-like	De-repressed AmpC	MBL
N	910	183	608	18	30	45	26
MIC_{50} (mg/L)	0.125	0.125	0.125	0.25	0.5	0.25	0.5
MIC_{90} (mg/L)	0.5	0.25	0.25	2	2	1	8

Isolates with Lower Susceptibility to Cefpodoxime-ETX1317

- 12 isolates with MIC values of 4 mg/L or greater were subjected to whole genome sequencing.
  - 5 *K. pneumoniae*, 2 *C. freundii*, 2 *E. cloacae*, and 3 *P. mirabilis*
- 8 of the 12 isolates were found to encode for a metallo-β-lactamase (NDM-1 or VIM). These isolates were confirmed to be carbapenem-resistant, suggesting robust expression of the MBL.
  - Result is predictable because ETX1317 does not inhibit Class B metallo-β-lactamases.
  - Investigation of the mechanism of resistance in the other 4 isolates is in progress.

Conclusions

- ETX0282 is an oral prodrug which is hydrolyzed *in vivo* to release ETX1317, a novel diazabicyclooctenone inhibitor of serine β-lactamases with activity against Ambler classes A and C enzymes and select class D enzymes.
- ETX0282 is being developed in combination with cefpodoxime proxetil, which is hydrolyzed *in vivo* to release cefpodoxime.
- The MIC_{90} of cefpodoxime against 910 recent, globally diverse ESBL-enriched *Enterobacteriaceae* from 2013-2015 was reduced from >32 to 0.5 mg/L when titrated in a 1:2 ratio with ETX1317.
- This potency was maintained across bacterial species and all β-lactamase classes associated with *Enterobacteriaceae* except for Class B MBLs.
- CPDP-ETX0282, represents the first, new oral β-lactam/β-lactamase inhibitor combination as a therapeutic option for the treatment of resistant Gram-negative uropathogens in decades.

References

1. Foxman, B. 2014. *Infect. Dis. Clin. North Amer.* 28: 1-13. **2.** Zowawi, H.M. *et al. Nat. Rev. Urol.* 2015. 12:570-84 **3.** CLSI M07-A10. 2015. **4.** CLSI M100, 28th ed. 2018.

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