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The Novel β-lactamase Inhibitor ETX1317 Restores the Activity of Cefpodoxime Against Drug-Resistant *Enterobacteriaceae*

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Abstract

Background
The treatment of infections caused by Gram-negative organisms has been complicated by the emergence of multi-drug resistant, β-lactamase (BLA)-expressing pathogens. ETX0282 is an oral prodrug which is actively metabolized *in vivo* to release ETX1317, a novel diazabicyclooctenone β-lactamase inhibitor active against serine BLAs. ETX0282 is currently in clinical development with cefpodoxime proxetil (CPDP), a clinically approved oral antibiotic which is hydrolyzed *in vivo* to release cefpodoxime (CPD). We sought to determine the *in vitro* antibacterial activity of CPD-ETX1317 against *Enterobacteriaceae* isolates expressing a variety of clinically-relevant drug-resistant phenotypes.

Methods
The minimal inhibitory concentration (MIC) for each strain was determined following Clinical and Laboratory Standards Institute (CLSI) guidelines. CPD and ETX1317 were tested individually and in combination at a 1:2 ratio. *Enterobacteriaceae* isolates tested included (1) the CDC and FDA Antibiotic Resistance Isolate Bank Carbapenemase Diversity Panel, each expressing a variety of resistance genes, including carbapenemases; (2) prevalent drug-resistant sequence types of *Escherichia coli* (ST131) and *Klebsiella pneumoniae* (ST258); and (3) *mcr-1+ E. coli*. Additionally, an isogenic panel of *E. coli* strains individually overexpressing a representative BLA from each Ambler class (A, B, C and D) was tested.

Results
ETX1317 restored the activity of CPD against all BLAs expressed in an *E. coli* isogenic panel, except for the class B metallo-β-lactamase (MBL) producing strains. ETX1317 also restored CPD activity against the Carbapenemase Diversity Panel from the CDC (CPD-ETX1317 MIC_{90} of 0.5 mg/L, N=30 non-MBLs). CPD-ETX1317 MIC values versus 13 *E. coli* ST131 CPD-resistant clinical isolates ranged from 0.12 – 1 mg/L. Similarly, the addition of ETX1317 to CPD restored susceptibility to 13 *K. pneumoniae* CC11/ST258 isolates, with MIC values ranging from 0.12 – 2 mg/L. MIC values for CPD-ETX1317 against 6 different *E. coli* isolates encoding *mcr-1*, which confers resistance to colistin, ranged from 0.06 - 0.25 mg/L.

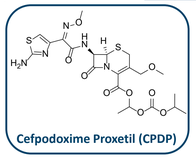
Conclusions
The combination of CPD and ETX1317 demonstrated potent antibacterial activity against a diverse set of *Enterobacteriaceae* expressing a variety of antibiotic-resistance genes. These data support the continued development of the oral combination of ETX0282 and CPDP for the treatment of antibiotic-resistant *Enterobacteriaceae* infections.

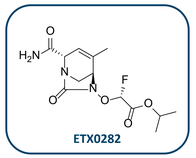
Introduction

Most urinary tract infections (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[2]. Resistance to existing oral therapies for UTI is forcing physicians to 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 actively metabolized *in vivo* to cefpodoxime (CPD) and ETX1317. The intended use for this product is as first line treatment for cUTI and pyelonephritis to avoid hospital admission, and as an oral switch/ stepdown for accelerated hospital discharge.

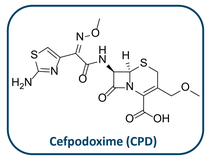
A Broad-spectrum Oral β-lactam/β-lactamase Inhibitor Combination

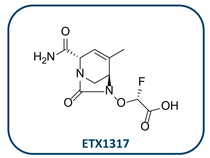
ETX0282-cefpodoxime proxetil is an **oral prodrug** combination:


Cefpodoxime Proxetil (CPDP)


ETX0282

The oral combination is metabolized *in vivo* to release **the active moieties** cefpodoxime-ETX1317:


Cefpodoxime (CPD)


ETX1317

ETX1317 is a β-lactamase inhibitor (BLI) that inhibits class A, C and many class D β-lactamases. ETX1317 also has intrinsic antibacterial activity against some *Enterobacteriaceae* but could not be considered as a stand-alone agent due to spectrum and spontaneous frequency of resistance.

ETX1317 Restores Cefpodoxime Antibacterial Activity against Class A, C and D β-lactamases Expressed in an Isogenic Panel of *E. coli*

Individual β-lactamase genes were constitutively expressed from a plasmid in an *E. coli* W3110 background. Clinically relevant examples of each of the Ambler class of β-lactamases (A, B, C and D) were tested for susceptibility to CPD-ETX1317 and comparator antimicrobial agents.

Ambler Class	β-lactamase content[a]	MIC (mg/L)[b]				
		PIP	TZP	ETX1317	CPD	CPD-ETX1317
None	*E. coli* W3110 Parent	4	4	16	2	0.12
A	CTX-M-15	64	4	16	>64	0.12
	TEM-1	>64	>64	16	1	0.12
	KPC-2	>64	>64	16	>64	0.25
	KPC-3	64	16	16	32	0.12
	SHV2a	>64	4	16	>64	0.12
	PER-1	8	2	8	32	0.12
	VEB-1	32	4	16	>64	0.25
	GES-11	4	4	16	8	0.12
B	NDM-1	64	64	16	>64	8
	VIM-1	>64	>64	16	>64	8
C	AmpC[c]	64	4	16	>64	0.25
	P99[d]	64	4	16	>64	0.25
D	OXA-1	>64	>64	16	8	0.25
	OXA-10	>64	64	4	2	0.12
	OXA-23	>64	64	16	1	0.12
	OXA-40	64	32	8	1	0.12
	OXA-48	>64	>64	16	2	0.12
	OXA-58	>64	>64	16	1	0.12

ETX1317 restored activity to CPD against Ambler class A, C and D β-lactamases.

ETX1317 did not restore CPD activity to parental levels when class B metallo β-lactamases (MBLs) were expressed.

[a]Each β-lactamase was expressed from plasmid pBBR1-MCS2. [b]CPD = cefpodoxime; CPD-ETX1317 = cefpodoxime titrated with ETX1317 in a 1:2 ratio; PIP = piperacillin; TZP = piperacillin + 4 mg/L tazobactam. [c]AmpC from *Pseudomonas aeruginosa*. [d]P99 from *Enterobacter cloacae*.

Materials and Methods

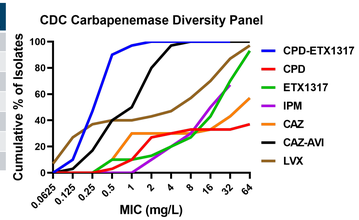
Broth microdilution susceptibility testing was conducted according to CLSI guidelines[3]. Where indicated, CPD-ETX1317 = MIC of cefpodoxime titrated with ETX1317 in a 1:2 ratio (a testing paradigm to minimize contributions of ETX1317 intrinsic antibacterial activity and maximize ETX1317 BLI activity). The *mcr-1+ E. coli* were a kind gift from P. Nordmann and L. Poirel (University of Fribourg, Switzerland). ST131 *E. coli* and CC11 *K. pneumoniae* were purchased from JMI Laboratories (North Liberty, IA, USA).

ETX1317 Restores Cefpodoxime Antibacterial Activity against Carbapenemase-Expressing *Enterobacteriaceae*

Activity of CPD-ETX1317 was tested against 30 *Enterobacteriaceae* isolates from the CDC & FDA Antibiotic Resistance (AR) Isolate Bank Carbapenemase Diversity Panel. Isolates were chosen to represent a diversity of species and carbapenemases (non-MBL isolates only).

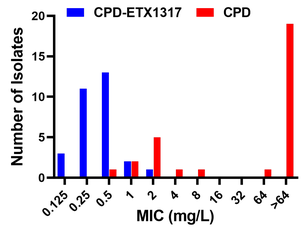
Composition of CDC & FDA *Enterobacteriaceae* Carbapenemase Diversity Panel (non-MBL only):

Bacterial Species	No. of Isolates	Carbapenemase	No. of Isolates
Citrobacter freundii	1	KPC-2	6
Enterobacter cloacae complex	4	KPC-3	11
Escherichia coli	1	KPC-6	1
Klebsiella oxytoca	1	NMC-A	2
Klebsiella pneumoniae	12	OXA-48	1
Kluyvera ascorbata	1	OXA-181	3
Morganella morganii	1	SME-3	6
Proteus mirabilis	2		
Raoultella ornithinolytica	1		
Serratia marcescens	6		



Antimicrobial Agent[a]	mg/L			CLSI[b]		
	MIC_{50}	MIC_{90}	Range	%S	%I	%R
CPD-ETX1317	0.5	0.5	0.12 - 2			
CPD	>64	>64	0.5 - >64	26.7	3.3	70
ETX1317	32	64	0.5 - >64			
IPM	16	>32	2 - >32	0	10	90
CAZ	64	>64	0.5 - >64	30	0	70
CAZ-AVI	1	4	0.12 - 8	100	0	0
LVX	8	64	0.06 - >64	40	0	60

[a]CPD = cefpodoxime; IPM = imipenem; CAZ = ceftazidime; CAZ-AVI = ceftazidime + 4 mg/L avibactam; LVX = levofloxacin. [b]Based on 2019 CLSI breakpoint criteria[4].



The presence of ETX1317 decreased the CPD MIC_{90} from >64 mg/L to 0.5 mg/L, a >128-fold reduction.

CPD-ETX1317 is Active against *mcr-1+ E. coli*

A troubling antibiotic resistance mechanism has recently emerged in the form of *mcr-1*, encoded on plasmids mediating colistin resistance[5]. Six *E. coli* isolated from animal waste and identified as carrying *mcr-1* were tested for sensitivity to CPD-ETX1317 and comparator antibiotics.

E. coli Isolate	MIC (mg/L)[a]					
	COL	IPM	CAZ-AVI	ETX1317	CPD	CPD-ETX1317
CDF2 (*mcr-1+*)	8	0.12	0.25	0.25	>64	0.12
CDF4 (*mcr-1+*)	4	0.25	0.25	4	>64	0.12
CDF8 (*mcr-1+*)	4	0.5	0.25	0.5	>64	0.25
A923 (*mcr-1+*)	4	0.12	0.12	2	1	0.06
A931 (*mcr-1+*)	4	0.12	0.12	8	0.5	0.12
A948 (*mcr-1+*)	4	0.5	0.25	4	>64	0.25

CPD-ETX1317 is active against colistin-resistant *mcr-1+ E. coli*.

[a]CPD = cefpodoxime; COL = colistin: IPM = imipenem; CAZ = ceftazidime; CAZ-AVI = ceftazidime + 4 mg/L avibactam.

Activity of Cefpodoxime-ETX1317 against *E. coli* ST131

A set of 13 *E. coli* ST131 (CC131) clinical isolates were obtained from JMI Laboratories:
- Sequence type associated globally with urinary, bladder and kidney infections and urosepsis
- Frequently fluoroquinolone-resistant and Extended-Spectrum β-lactamase (ESBL) - producing

Antimicrobial Agent[a]	mg/L			CLSI[b]		
	MIC_{50}	MIC_{90}	Range	%S	%I	%R
CPD-ETX1317	0.12	0.5	0.12 - 1			
CPD	>64	>64	0.5 - >64	7.7	0	92.3
ETX1317	0.5	1	0.25 - 2			
IPM	0.12	0.12	0.06 - 0.25	100	0	0
CAZ	16	>64	0.5 - >64	15.4	7.7	76.9
CAZ-AVI	0.5	1	0.12 - 2	100		0
TZP	4	64	1 - >64	76.9	15.4	7.7
LVX	16	32	0.5 - 64	7.7	0	92.3
SXT	0.5	>8	0.06 - >8	61.5		38.5

CPD-ETX1317 is active against ST131 *E. coli*.

[a]CPD = cefpodoxime; IPM = imipenem; CAZ = ceftazidime; CAZ-AVI = ceftazidime + 4 mg/L avibactam; TZP = piperacillin + 4 mg/L tazobactam; LVX = levofloxacin; SXT = trimethoprim-sulfamethoxazole (1:19) . [b]Based on 2019 CLSI breakpoint criteria[4].

Activity of Cefpodoxime-ETX1317 against *K. pneumoniae* ST258

A set of 13 *K. pneumoniae* CC11 clinical isolates were obtained from JMI Laboratories that represent clinically predominant sequence types:
- ST258 (n=9); ST340 (n=1), ST11 (n=1) and ST1326 (n=1)
- ST258 is a predominant KPC-encoding lineage in the USA and other parts of the world. ST258 is associated with MDR phenotypes and frame-shift mutations in the *ompK35* outer membrane porin gene.

Antimicrobial Agent[a]	mg/L			CLSI[b]		
	MIC_{50}	MIC_{90}	Range	%S	%I	%R
CPD-ETX1317	0.5	1	0.12 - 2			
CPD	>64	>64	64 - >64	0	0	100
ETX1317	16	32	0.5 - 64			
IPM	16	>32	0.12 - >32	15.4	15.4	69.2
CAZ	>64	>64	32 - >64	0	0	100
CAZ-AVI	4	16	1 - >64	76.9		23.1
TZP	>64	>64	8 - >64	7.7	0	92.3
LVX	64	64	8 - >64	0	0	100
SXT	>8	>8	0.5 - >8	7.7		92.3

CPD-ETX1317 is active against CC11 *K. pneumoniae*, including ST258

[a]CPD = cefpodoxime; IPM = imipenem; CAZ = ceftazidime; CAZ-AVI = ceftazidime + 4 mg/L avibactam; TZP = piperacillin + 4 mg/L tazobactam; LVX = levofloxacin; SXT = trimethoprim-sulfamethoxazole (1:19) . [b]Based on 2019 CLSI breakpoint criteria[4].

Conclusions

- ETX0282 is an oral prodrug which is metabolized *in vivo* to release ETX1317, a novel diazabicyclooctenone inhibitor of serine β-lactamases with activity against Ambler classes A, C and many class D enzymes.
- ETX0282 is being developed in combination with oral cefpodoxime proxetil, which is metabolized *in vivo* to release cefpodoxime.
- Cefpodoxime-ETX1317 has potent *in vitro* activity against a variety of clinically-relevant drug-resistant phenotypes, including carbapenem and colistin resistance, as well as MDR sequence types of *E. coli* and *K. pneumoniae*.
- These data support the continued development of the oral combination of ETX0282 and CPDP for the treatment of antibiotic-resistant *Enterobacteriaceae* infections.

References

1. Foxman, B. (2014) *Infect. Dis. Clin. North Amer.* 28: 1-13. **2.** Zowawi, H.M. *et al.* (2015) *Nat. Rev. Urol.* 12:570-84 **3.** CLSI M07-A10. 2015. **4.** CLSI M100, 29th ed. 2019. **5.** Liu, Y.Y. *et al.* (2016) *Lancet Infect. Dis.* 16: 161-168.

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