Session-198
SATURDAY-278

Cefpodoxime proxetil/ETX0282: A novel oral β-lactam/β-lactamase inhibitor combination to treat the emerging threat of multi-drug resistant *Enterobacteriaceae*

J. O'Donnell, A. Chen, A. Tanudra, J. Mueller, R. Tommasi, A. Miller, N. Carter, S. McLeod, J. Comita-Prevoir and T. Durand-Réville
Entasis Therapeutics, Waltham, MA, USA

Entasis Therapeutics
1-781-810-0118
www.entasistx.com

Abstract

Background
Multi-drug resistant (MDR) *Enterobacteriaceae* expressing a wide array of β-lactamases are emerging as a health threat not only in the hospital, but in the community as well. ETX0282 is a novel, orally available prodrug ester of a novel diazabicyclooctenone β-lactamase inhibitor, ETX1317, which has broad spectrum activity against Ambler classes A, C and D serine β-lactamases. ETX1317 successfully restores the activity of cefpodoxime (CPD) against MDR enteric isolates including KPC-producing carbapenem-resistant *Enterobacteriaceae* (CRE) strains. The attributes of ETX0282 in combination with prodrug cefpodoxime proxetil (CPDP) are discussed.

Methods
Oral (PO) and intravenous (IV) PK of ETX0282 and ETX1317, respectively, were completed in rats and dogs to determine bioavailability. *In vitro* stability of ETX0282 was assessed in rat, dog, and human intestinal and liver S9 subcellular fractions and formation of ETX1317 was monitored by LC/MS/MS. Efficacy of ETX0282 in combination with CPDP was investigated using two CPD insensitive clinical isolates (MIC > 64 mg/L) in an in vivo neutropenic thigh model. Efficacy was determined as the change in CFU over the course of the study.

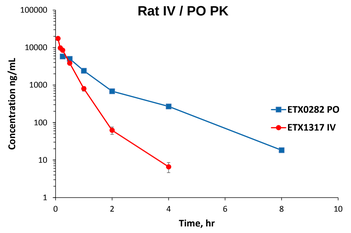
Results
Rat and dog PO/IV PK studies with ETX0282 and ETX1317 suggested bioavailability in excess of 90% for both species and an elimination half-life similar to CPD. Liver S9 demonstrated more rapid and quantitative conversion of ETX0282 to ETX1317 as compared to intestine across all species, suggesting first pass conversion of prodrug to active occurs predominantly in the liver. A dose dependent reduction in CFUs was observed following administration of 50 mg/kg of CPDP and increasing doses of ETX0282 with nearly 1-log kill achieved in the neutropenic thigh model. Administration of ETX0282 or CPDP alone was not efficacious.

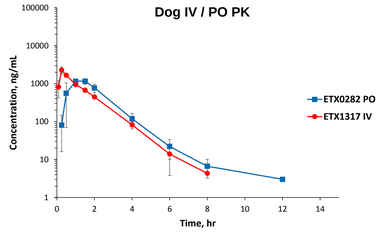
Conclusions
The prodrug ETX0282 demonstrates high bioavailability of ETX1317 following oral administration in preclinical species with similar ADME attributes as CPDP. Oral murine efficacy studies with CPDP/ETX0282 shows promising activity warranting further clinical evaluation of the combination.

Introduction
ETX0282 is an orally bioavailable prodrug ester of the novel β-lactamase inhibitor ETX1317 that is targeted for use in combination with cefpodoxime proxetil (CPDP). ETX1317 effectively restores the MIC potency of cefpodoxime against the increasingly prevalent fluoroquinolone resistant *Enterobacteriaceae* isolates associated with complicated urinary tract infections. Potency is not only achieved against ESBL producing isolates, but the combination also holds promising activity against KPC producing Carbapenem-Resistant *Enterobacteriaceae* as demonstrated in recent surveillance MIC data (McLeod *et al*. poster 279).
The present study summarizes the non-clinical pharmacokinetic parameters of ETX1317 and its oral prodrug ester ETX0282. Conversion efficiency as assessed in liver S9 sub-cellular fractions across non-clinical species in addition to human were utilized in support of prodrug optimization and selection. Demonstration of oral efficacy of CPDP/ETX0282 against relevant clinical isolates including CRE strains in a murine neutropenic thigh model provides the critical evidence for activity required to be used in conjunction with future PK/PD studies in support of a human dose projection.

Methods
IV/PO pharmacokinetics in rats and dogs: Single dose IV and PO pharmacokinetics of ETX1317 and ETX0282, respectively were conducted in n=3 male Sprague-Dawley rats (200 gm) and n=3 male beagle dogs at 10 and 1 mg/kg respectively (active eq. dose in PO). Doses were dissolved in 25:75 PEG400:DI water (pH 4.7). Serial blood samples were obtained via venipuncture, prepared for plasma and assayed for drug concentrations by LC/MS/MS. ETX1317 concentrations were assayed in plasma and urine to determine clearance mechanism. Pharmacokinetic parameters were determined by non-compartmental analysis using Phoenix WinNonLin 6.4 software.
In Vitro S9 Stability: Liver S9 sub-cellular fractions (Xenotech) from rat, dog, and human tissue were diluted to a protein concentration of 0.8 mg/mL in 100 mM potassium phosphate buffer, pH 7.4 and pre-incubated in a 37C water bath for 5 minutes prior to addition of 10 µM (final) of ETX0282. Serial aliquots were removed at 0, 2, 5, 10, 20, 40, and 60 minutes and quenched in acetonitrile with internal standard prior to LC/MS/MS to determine ETX0282 and ETX1317 concentrations. First order degradation half-lives were determined from the slope of the log–linear plots of the depletion data.
In Vivo Mouse Infection Models: Mice were rendered neutropenic with cyclophosphamide and infected with selected clinical *E.coli* and *K. pneumoniae* isolates via a thigh abscess model. Infected mice were treated via oral administration 2 hours post bacterial inoculation with either ETX0282, CPDP, or CPDP in combination with ETX0282. CPDP doses were given at a constant 50 mg/kg dose and a dose titration of ETX0282 was completed in the presence of CPDP. All doses were administered using a q6h regimen. Efficacy was determined as the change in viable bacterial counts in tissue 24 hours after start of treatment. Plasma exposures of ETX1317 and cefpodoxime were determined by LC/MS/MS. A standard 50 mg/kg q6h dose of CPDP in mice provided an equivalent exposure as the 400 mg clinical dose of CPDP. The meropenem 600 mg/kg q6h SC mimics clinical exposure of 1 gm q8h.

Rat and Dog Pharmacokinetics and *In Vitro* S9 Incubations





ETX1317 Intravenous Pharmacokinetics

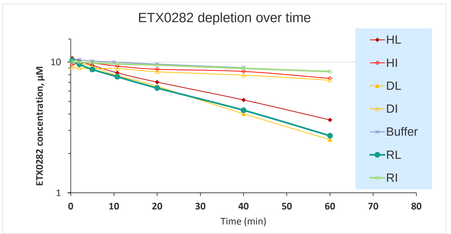
Species	Dose (mg/kg)	C_{max} (µg/mL)	AUC (µg.h/mL)	$T_{1/2}$ (hr)	Vd_{ss} (L/kg)	CL (mL/min/kg)	Renal CL (mL/min/kg)
Rat	10	17.5±2.0	7.19±0.34	0.4±0.02	0.70±0.01	23.2±1.1	ND
Dog	1	2.27±0.49	2.76±0.34	0.8±0.1	0.41±0.03	5.7±0.7	1.1±0.3

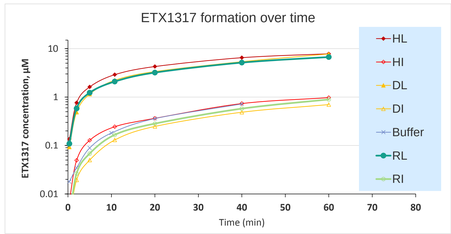
ETX0282 Oral Pharmacokinetics

Species	Dose (mg/kg)	C_{max} (µg/mL)	AUC (µg.h/mL)	$T_{1/2}$ (hr)	F%
Rat	10 equiv.	5.81±0.16	7.04±0.62	1.1±0.3	98
Dog	1 equiv.	1.27±0.02	2.68±0.24	1.3±0.6	97

ETX0282 *In Vitro* Intestinal and Liver S9 Stability

$T_{1/2}$ in minutes						
Buffer pH 7.4	Rat Intestinal S9	Rat Liver S9	Dog Intestinal S9	Dog Liver S9	Human Intestinal S9	Human Liver S9
186	240	32	186	30	163	39



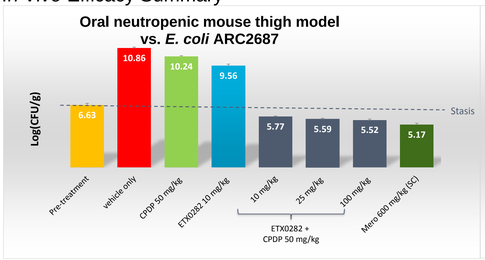


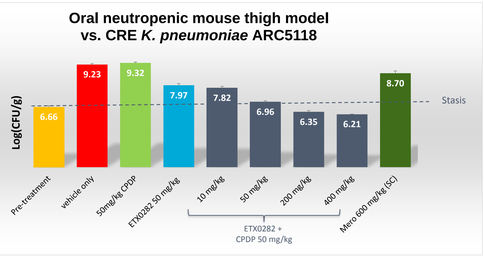
In Vivo Efficacy

MICs of PK/PD Sentinel Strains

Strain ID	Species	β-lactamase content	MIC (mg/L)				
			CPD	ETX1317	CPD + 4 mg/L ETX1317	CPD:ETX1317 (1:1 ratio)	Meropenem
ARC2687	*E. coli*	Amp C, CTX-M-14	>64	0.5	≤0.03	0.25	0.03
ARC4488	*K. pneumoniae*	SHV-11, CTX-M-15, OXA-1, TEM-1	>64	0.5	≤0.03	0.5	0.06
ARC5118	*K. pneumoniae*	SHV-5, TEM-1, KPC-3	>64	32	0.125	ND	>64
ARC5289	*K. pneumoniae*	SHV-1, KPC-2	>64	16	≤0.06	0.5	>64

In Vivo Efficacy Summary





Group	Dose (mg/kg)	Regimen	Log_{10}CFU/gm thigh change 24 hours post R_x initiation			
			ARC2687	ARC4488	ARC5118	ARC5289
Time =0 Rx	n/a	PO/q6h	--	--	--	--
Infection control	vehicle	PO/q6h	+4.59	+4.12	+2.57	+2.56
CPDP only	50	PO/q6h	+3.97	+3.58	+2.66	+2.60
ETX0282 only	50	PO/q6h	+3.29 (10 mg/kg)	ND	+1.31	+2.21
ETX0282:CPDP	10 : 50	PO/q6h	-0.50	+0.74	+1.16	+1.58
	50 : 50	PO/q6h	-0.68 (25 mg/kg)	-0.18	+0.30	+0.71
	200 : 50	PO/q6h	-0.75 (100 mg/kg)	-0.43	-0.31	-0.14
	400 : 50	PO/q6h	ND	ND	-0.38	ND
Meropenem	600	SC/q6h	-1.10	ND	+2.04	+1.31

Conclusions
- **The prodrug ester ETX0282 demonstrates high bioavailability in rats and dogs following oral administration.**
- **Intravenous kinetics in rats and dogs are consistent with cefpodoxime suggesting favorable attributes as a fixed dose combination.**
- **Oral efficacy of ETX0282 + cefpodoxime proxetil in a mouse neutropenic thigh model suggests cidal activity can be achieved against clinical isolates including Carbapenem Resistant *Enterobacteriaceae*.**
- **Efficacy was not observed with ETX1317 dosed alone even in instances when systemic concentrations exceeded its MIC.**

Acknowledgements: *In vivo* efficacy support was provided by Neosome Life Sciences, Lexington MA

Presented at Microbe 2017, New Orleans, LA June 2017