ASM Microbe 2019
SUNDAY – AAR-727

Assessment of the *In Vivo* Pharmacodynamic Profile of Ceftibuten (CTB)/VNRX-7145 Combination against Serine β-Lactamase-Producing Enterobacteriaceae (SBL-EB) in the Neutropenic Murine Thigh Infection Model

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ABSTRACT (revised)

Background: There are limited oral antibiotics for outpatient treatment of urinary tract infections caused by SBL-EB, especially carbapenemase (eg, KPC, OXA-48) producers. VNRX-7145 is an orally bioavailable pro-drug of VNRX-5236, a novel boronic acid-based β-lactamase inhibitor that lacks inherent antibacterial activity. VNRX-5236 is capable of restoring *in vitro* activity to CTB, an oral cephalosporin, in the presence of SBLs; however the *in vivo* pharmacodynamic profile of CTB/VNRX-5236 combination is undefined.

Methods: CTB-resistant SBL-EB with CTB/VNRX-5236 MIC range 0.12–2 μg/mL (VNRX-5236 fixed at 4 μg/mL) were assessed (N=21), including 4 carbapenem-hydrolyzing OXA-positive and 5 KPC-positive strains. Thigh infections in neutropenic ICR mice were established via intramuscular injection with bacterial suspensions of ~10^7 CFU/mL. A CTB human-simulated regimen (HSR), equivalent to a 300 mg q8h dosage, was developed in the murine infection model. In dose fractionation studies against 6 of the 21 isolates, animals received CTB HSR in combination with selected total daily dosages of VNRX-5236 (range: 1.2–38 mg/kg/day) as a single dose (q24h), q12h, or q6h. In dose ranging studies, CTB HSR was administered alone or in combination with 4 escalating VNRX-5236 exposures (CTB:VNRX-5236 dose ratio: 10:1, 3:1, 1:1, and 1:3). Efficacy was assessed as change in $\log_{10}$ CFU/thigh at 24 h compared with 0 h controls. The VNRX-5236 exposure required to achieve stasis against each isolate was estimated with the Hill equation.

Results: For the same total daily dose, reductions in bacterial burdens at 24 h were comparable irrespective of the VNRX-5236 dosing frequency indicating that the free area under the concentration-time curve to MIC ratio (*f*$AUC_{0\text{-}24}$/MIC) was the driver of VNRX-5236 activity. In dose ranging studies, compared with 0 h controls (mean $\log_{10}$ CFU/thigh, 5.8 ± 0.3), all isolates demonstrated growth in control (saline-dosed) and CTB HSR groups with mean increases of 3.0 ± 0.7 and 2.2 ± 0.8 $\log_{10}$ CFU/thigh, respectively. The addition of VNRX-5236 resulted in bacterial stasis in all isolates; the mean reduction with the 1:1 CTB:VNRX-5236 dose ratio was -0.6 ± 0.5 $\log_{10}$ CFU/thigh. The median *f*$AUC_{0\text{-}24}$/MIC associated with a static endpoint was 9.0 (range: 0.1**–**193.6).

Conclusions: These data demonstrate *in vivo* activity of CTB/VNRX-5236 against SBL-EB, including carbapenemase producers, and support *f*$AUC_{0\text{-}24}$/MIC as the PK/PD driver of VNRX-5236 efficacy. The identified efficacy target should guide Phase 2 dose selection.

INTRODUCTION

- Periods of increased hospitalizations for urinary tract infection (UTI) coincide with reports of increasing rates of resistance to first-line outpatient antibiotics.[1]
- Currently, there are limited oral antibiotic options when treating UTIs caused by ESBL-, OXA-48-, and KPC-positive Enterobacteriaceae (SBL-EB).[1-2]
- VNRX-7145 is a novel, orally bioavailable boronic acid-based β-lactamase inhibitor that lacks intrinsic antibacterial activity.
- VNRX-5236, the active moiety of the VNRX-7145 pro-drug, has been shown to enhance the *in vitro* activity of ceftibuten, an oral cephalosporin, against ESBL-, OXA-48-, and KPC-positive Enterobacteriaceae.

OBJECTIVE

- To determine and quantify the pharmacokinetic/pharmacodynamic (PK/PD) index associated with *in vivo* efficacy of VNRX-5236 in combination with ceftibuten (CTB) against CTB-resistant Enterobacteriaceae isolates expressing various serine-β-lactamases in the murine thigh infection model.

MATERIALS & METHODS

- VNRX-5236 (Batch no. YD00113-010, VenatoRx Pharmaceuticals, Inc., Malvern, PA) and ceftibuten (Batch no. CHBX170002, Covalent Laboratories Pvt. Ltd., Telangana, India) were used for all *in vivo* and *in vitro* studies.
- Clinical Enterobacteriaceae isolates were provided by the sponsor or previously indexed into the laboratory isolate library.
- CTB MICs were determined in triplicate by broth microdilution,[3] and CTB/VNRX-5236 MICs were performed in triplicate with a fixed VNRX-5236 concentration of 4 μg/mL.

MATERIALS & METHODS (continued)

Pharmacokinetic Analyses

- PK studies of CTB were undertaken to identify a regimen that provided mouse plasma exposures similar to those achieved in humans following a clinical dose of 300 mg when given every 8 hours (i.e., the CTB human-simulated regimen, CTB HSR).[4,5]
- Infected mice (n=36) were administered CTB and VNRX-5236 and groups of 6 mice were euthanized via CO_2-asphyxiation at 6 time points prior to blood collection by cardiac puncture.
- Ceftibuten PK studies were conducted with and without concomitant administration of VNRX-5236 to confirm that the target %*f*T>MIC exposures were achieved (**Table 1**).
- VNRX-5236 single-dose PK studies were carried out in infected mice (n=48) with 6 mice comprising each of 8 time points; the single-doses (1.2, 12, 38 mg/kg) were administered with the confirmed CTB HSR.
- Concentrations were determined using a LC-MS/MS method; PK and PD models were built in Phoenix WinNonlin v8.1 (Certara, Inc., Princeton, NJ).

	%*f*T>MIC for a MIC (μg/mL) of:					
Species	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**
Human	100%	100%	100%	91%	59%	0%
Mouse (HSR)	100%	100%	100%	90%	60%	1%

Table 1. Target human %*f*T>MIC exposures at steady-state following administration of CTB 300 mg q8h and those achieved in mice administered the CTB HSR.

Neutropenic Murine Thigh Infection Model

- Specific-pathogen-free female ICR mice (20–22 g) were purchased from Envigo RMS, Inc. (Indianapolis, IN) and rendered neutropenic with cyclophosphamide 150 mg/kg (mean body weight) intraperitoneally (IP) at 4 days and 100 mg/kg one day prior to inoculation; uranyl nitrate (5 mg/kg IP) was administered 3 days prior to inoculation to induce renal impairment and assist with humanizing a ceftibuten regimen.
- Bacterial suspensions (~10^7 CFU/mL) for inoculation were prepared from a second subculture of previously frozen stock; mice were inoculated with 0.1 mL of the suspension via intramuscular injection into each thigh.

Dose Fractionation Studies

- A total of 6 ESBL-producing Enterobacteriaceae strains were assessed; two strains (EC C11-23 and EC 617) were also AmpC-positive.
- The CTB HSR was given in combination with 3 total daily VNRX-5236 doses (4 and 12 mg/kg/day, and either 1.2 or 38 mg/kg/day); each total daily dose was administered as a q24h, q12h, or q6h regimen.
- Comparisons of bacterial burdens at 24 h were made between the different regimens of the same total daily dose using one-way analysis of variance (ANOVA) followed by Tukey's test with a p-value of < 0.05 (SigmaPlot, Systat Software, Inc., San Jose, CA).

Dose Ranging Studies

- Efficacy of CTB HSR in combination with escalating VNRX-5236 dosages was assessed against CTB-resistant SBL-EB (N=21).
- Efficacy was measured as the change in $\log_{10}$ CFU/thigh at 24 h compared with 0 h controls.
- Pharmacokinetics of VNRX-5236 were assessed to determine the exposures of the regimens utilized; exposures required to achieve efficacy endpoints were estimated using the Hill equation.

RESULTS

Susceptibility Testing and Pharmacokinetic Analyses

- For all isolates, the CTB MIC was ≥ 32 μg/mL, while CTB/VNRX-5236 MICs ranged from 0.12 to 2 μg/mL (**Table 2**).
- The CTB HSR that mimics the human profile was a 3-dose regimen, 1.5, 1.3, and 1.2 mg/kg at 0-h, 2.25-h, and 4.75-h, respectively, repeated every 8 hours; concomitant VNRX-5236 had no effect on the PK of CTB *in vivo*.
- CTB *f*AUC and fC_{max} were also similar in humans versus mouse: 56.2 versus 56.5 μg · h/mL, and 3.8 versus 4.0 μg/mL, respectively.

Pharmacodynamic Assessments

- Fractionation of VNRX-5236 exposures (**Figure 1**) indicated that the efficacy of the inhibitor was not associated with %*f*T>MIC or fC_{max}/MIC as the dosing frequency did not impact the extent of VNRX-5236 potentiation of CTB activity.
- In dose ranging studies, bacterial stasis was achieved against all SBL-EB isolates (**Table 2, Figure 2**).

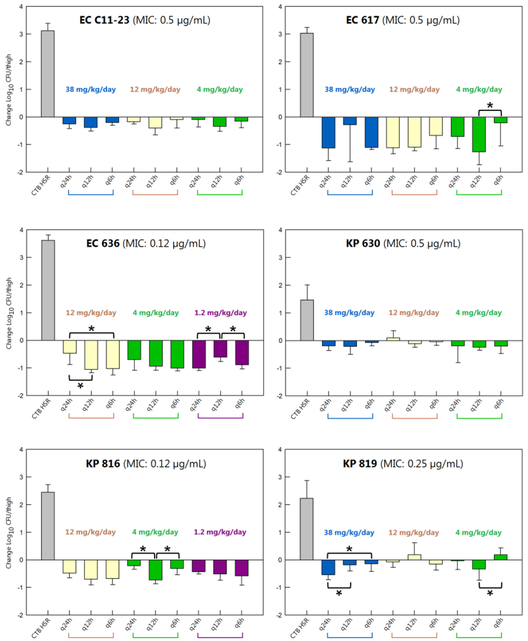

Figure 1. VNRX-5236 dose fractionation results. The MIC indicated in each panel is that of CTB/VNRX-5236 in the presence of a fixed VNRX-5236 concentration of 4 μg/mL. Post-hoc p-values < 0.05 are indicated with asterisks.

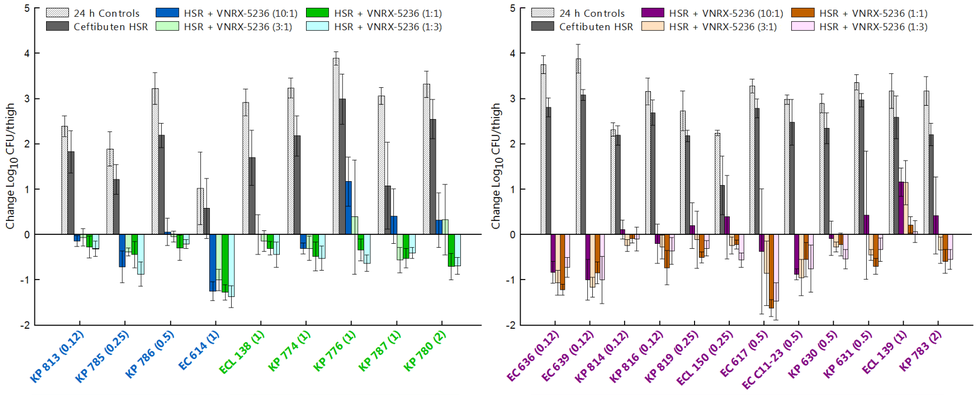

Figure 2. Change in $\log_{10}$ CFU/thigh (mean ± SD) at 24 h in a neutropenic murine thigh infection model. The modal CTB/VNRX-5236 combination MIC (VNRX-5236 fixed at 4 μg/mL) is listed in parentheses for each *Enterobacter cloacae* (ECL), *Escherichia coli* (EC), and *Klebsiella pneumoniae* (KP) isolate.

Table 2. Description of bacterial isolates and efficacy targets

Isolate (MIC, μg/mL)[a]	Encoded β-Lactamase(s)	VNRX-5236 *f*$AUC_{0\text{-}24}$/MIC[a] Required to Achieve Stasis	
		Target	R^2
EC 614 (1)	OXA-48	0.7	0.82
EC 617 (0.5)	AmpC, CTX-M-15, SHV-5, SHV-1, TEM-1	7.6	0.83
EC 636 (0.12)	CTX-M-15	18.9	0.97
EC 639 (0.12)	CTX-M-15, TEM-1	18.2	0.96
EC C11-23 (0.5)	AmpC, CTX-M-15, TEM-1	0.1	0.92
ECL 138 (1)	KPC	5.9	0.81
ECL 139 (1)	AmpC (p99), CTX-M-3, TEM-1	193.6	0.85
ECL 150 (0.25)	CTX-M-15	43.2	0.59
KP 630 (0.5)	CTX-M-15, SHV-WT, TEM-WT	1.5	0.93
KP 631 (0.5)	CTX-M-15, SHV-WT, TEM-WT	14.9	0.83
KP 774 (1)	KPC	0.6	0.92
KP 776 (1)	KPC	30.9	0.82
KP 780 (2)	KPC	9.0	0.84
KP 783 (2)	CMY-2, TEM-1	4.6	0.87
KP 785 (0.25)	OXA-204	0.1	0.84
KP 786 (0.5)	OXA-48	17.1	0.94
KP 787 (1)	KPC	6.4	0.63
KP 813 (0.12)	CTX-M-15, OXA-48, SHV-12, TEM-1	9.0	0.91
KP 814 (0.12)	SHV-12, TEM-1	15.8	0.95
KP 816 (0.12)	CTX-M-3, SHV-12, TEM-1	28.6	0.93
KP 819 (0.25)	SHV-12	13.8	0.93
	Median (interquartile range)	**9.0 (3.1–18.6)**	

[a] Based on the MIC of CTB/VNRX-5236 (VNRX-5236 concentration of 4 μg/mL)

CONCLUSIONS

- **The addition of VNRX-5236 potentiated the activity of ceftibuten against all CTB-resistant SBL-EB strains assessed.**
- **In combination with ceftibuten, the *f*$AUC_{0\text{-}24}$/MIC was the PK/PD index that appeared to drive *in vivo* activity of VNRX-5236.**
- **The median (range) exposure associated with bacterial stasis was a *f*$AUC_{0\text{-}24}$/MIC of 9.0 (0.1–193.6); these data should guide VNRX-5236 dose selection for Phase 2 studies to ensure human exposures are aligned with efficacious exposures utilized in pre-clinical studies.**

ACKNOWLEDGMENTS

- The authors thank Christina Sutherland, Sara Giovagnoli, Debora Santini, Jennifer Tabor-Rennie, Elizabeth Cyr, Kimelyn Greenwood, Alissa Padgett, Courtney Bouchard, Lauren McLellan, Elias Mullane, Michelle Insignares, Janice Cunningham, Nicole DeRosa, Oleysa Slipchuk, Charlie Cote, Sean Stainton, Safa Almarzoky Abuhussain, James Kidd, Tomefa Asempa, Sergio Reyes, and Iris Chen for their assistance in the conduct of this study.
- This project has been funded with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN272201600029C awarded to VenatoRx Pharmaceuticals, Inc., Malvern, PA.

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