Advanced preclinical *in vitro* and *in vivo* characterization of a novel, non-hydroxamate-based LpxC inhibitor for the intravenous and oral treatment of multidrug-resistant Enterobacterales

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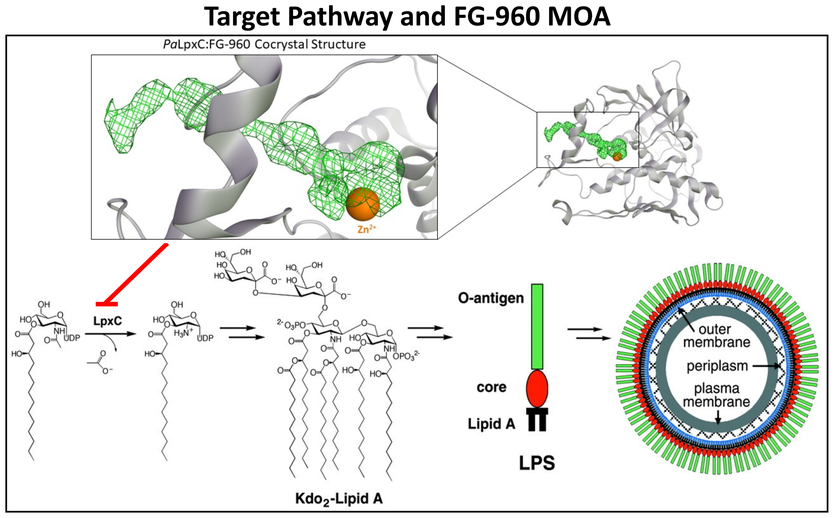
Abstract

LpxC is an essential metalloenzyme that Gram-negative bacteria require for outer membrane biosynthesis. Previous antibacterial efforts have focused on hydroxamate-based small molecules to engage LpxC's catalytic zinc. Unfortunately, these previous attempts have been hampered by safety liabilities. Leveraging its metalloenzyme inhibitor platform, Blacksmith has identified FG-960, a non-hydroxamate-based LpxC inhibitor with strong *in vitro* and *in vivo* profiles against MDR Enterobacterales, and is currently being advanced as a treatment for urinary tract infections (UTIs).

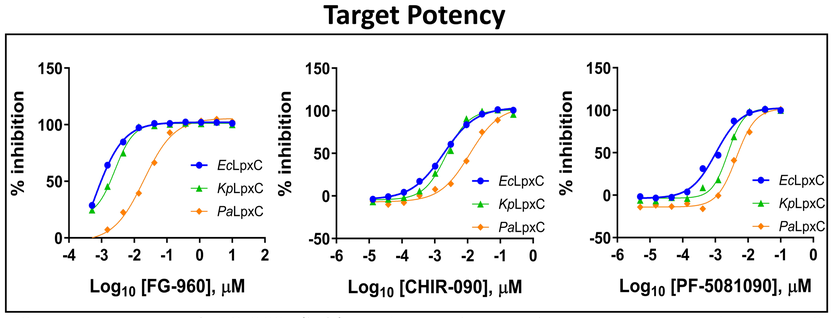
FG-960 is highly-potent against the LpxC enzyme (*E.coli* IC_{50} = 1 nM), and has strong whole cell activity against panels of contemporary MDR and non-MDR Enterobacterales isolates (MIC_{90} = 4 µg/ml; n= 973 strains). Static time kill studies demonstrate FG-960's rapid bactericidal activity, with regrowth suppression evident at concentrations ≥2X MIC. When evaluated in standard frequency of resistance assays, FG-960 shows spontaneous resistance emergence at frequencies consistent with historical LpxC inhibitors ($1x10^{-8}$-$1x10^{-10}$) at 4X MIC against multiple MDR and non-MDR strains. Pharmacokinetic studies have demonstrated dose-proportional exposures in the plasma from preclinical species, with considerable accumulation of intact FG-960 in the urinary tract. This higher localized exposure likely contributes to FG-960's strong *in vivo* efficacy profile, with >1-log kill achieved using either intravenous or oral administration at dose levels of 10-50 mg/kg/day in a mouse UTI model. Importantly, and in multiple preclinical models, FG-960 does not exhibit cardiovascular toxicity observed with previous hydroxamate-based LpxC inhibitors. In summary, Blacksmith is advancing FG-960 in ongoing IND-enabling studies for the treatment of UTIs caused by MDR Gram-negative bacteria.

Target Pathway and FG-960 MOA



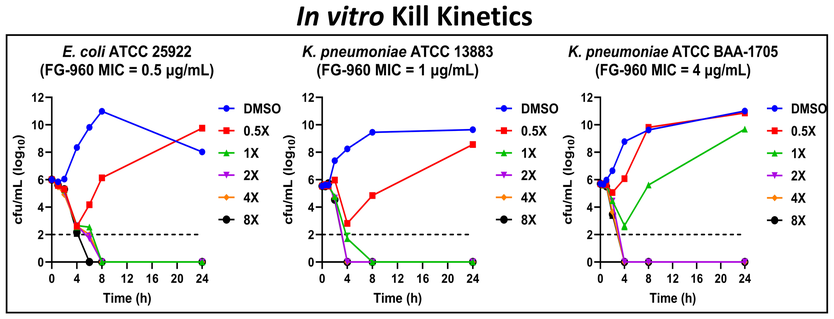
Role of LpxC in Gram-negative bacterial outer membrane biogenesis (adapted from Whittington *et al*, PNAS, July 2003), and the inhibitory mechanism of action for FG-960.

Target Potency



IC_{50} assessment of FG-960 (left) and two legacy, hydroxamate-containing LpxC inhibitors, CHIR-090 (middle) and PF-5081090 (right). Each compound was evaluated against purified LpxC orthologs from *E. coli* (*Ec*), *K. pneumoniae* (*Kp*), and *P. aeruginosa* (*Pa*). IC_{50} values were calculated as follows - FG-960: 1.05, 1.40, and 21.40 nM for *Ec*, *Kp*, and *Pa*; CHIR-090: 0.30, 0.6, and 11.3 nM; PF-5081090: 0.2, 0.5, and 2.2 nM.

In vitro Kill Kinetics



In vitro static time-kill results with FG-960 evaluated against three different Enterobacterales target strains. Compound was added to exponentially-growing cultures of each indicated isolate at the fold-MIC concentrations specified. Recoverable bioburdens were quantified via viable plate counting at 0, 1, 2, 4, 6, 8, and 24 hours post-compound addition.

In vitro Activity Spectrum (MIC_{90})

Antimicrobial agent	*E. coli* (n=108)		*K. pneumoniae* (n=107)		*K. oxytoca* (n=56)		*K. aerogenes* (n=52)		*P. mirabilis* (n=54)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
FG-960	0.5	1	0.5	4	1	2	0.5	2	1	4
Ceftazidime-avibactam	0.06	0.25	0.12	0.25	0.12	0.25	0.12	0.25	≤0.03	0.06
Ceftriaxone	0.06	>8	0.06	>8	0.06	>8	0.12	>8	≤0.004	0.008
Ciprofloxacin	≤0.06	64	≤0.06	64	≤0.06	0.25	≤0.06	0.12	≤0.06	4
Meropenem	0.015	0.03	0.03	0.06	0.03	0.03	0.03	0.06	0.12	0.12
Amoxicillin-clavulanate	8	16	2	16	2	16	32	>32	1	8
Nitrofurantoin	16	32	64	>64	32	64	64	>64	>64	>64
Piperacillin-tazobactam	2	16	4	32	2	128	4	32	0.25	1
Trimethoprim-sulfamethoxazole	≤0.12	>16	≤0.12	>16	≤0.12	4	≤0.12	0.25	≤0.12	>16

Antimicrobial agent	*C. freundii* (n=52)		*C. koseri* (n=54)		*E. cloacae* (n=52)		*M. morganii* (n=50)		*S. marcescens* (n=51)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
FG-960	1	4	0.5	1	1	2	2	4	0.5	1
Ceftazidime-avibactam	0.12	0.5	0.06	0.12	0.25	1	0.06	0.25	0.25	0.5
Ceftriaxone	0.25	>8	0.06	0.12	0.5	>8	0.06	4	0.5	>8
Ciprofloxacin	≤0.06	4	≤0.06	≤0.06	≤0.06	0.5	≤0.06	2	≤0.06	2
Meropenem	0.03	0.06	0.015	0.03	0.03	1	0.06	0.12	0.06	0.12
Amoxicillin-clavulanate	32	>32	2	4	>32	>32	>32	>32	>32	>32
Nitrofurantoin	32	32	64	>64	64	>64	64	>64	>64	>64
Piperacillin-tazobactam	4	128	2	4	4	>128	0.25	128	2	32
Trimethoprim-sulfamethoxazole	≤0.12	>16	≤0.12	≤0.12	≤0.12	>16	≤0.12	>16	0.5	4

MIC profiling of FG-960 against contemporary and geographically-distinct clinical isolates of Enterobacterales pathogens commonly found to cause UTIs.

Frequency and Mechanism of FG-960 Resistance

Compound	Fold MIC	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 13883
FG-960	4X	$1.83x10^{-10}$	$1.84x10^{-8}$
	8X	$<1.83x10^{-10}$	$1.35x10^{-9}$
	16X	$<1.83x10^{-10}$	$<2.70x10^{-10}$
ACHN-975	4X	$9.14x10^{-10}$	$2.05x10^{-8}$
	8X	$<1.83x10^{-10}$	$2.70x10^{-9}$
	16X	$<1.83x10^{-10}$	$<2.70x10^{-10}$
PF-5081090	4X	$1.33x10^{-8}$	Not Tested
	8X	$1.33x10^{-8}$	
Ciprofloxacin	4X	$3.66x10^{-10}$	
	8X	$1.10x10^{-9}$	
	16X	$<1.83x10^{-10}$	

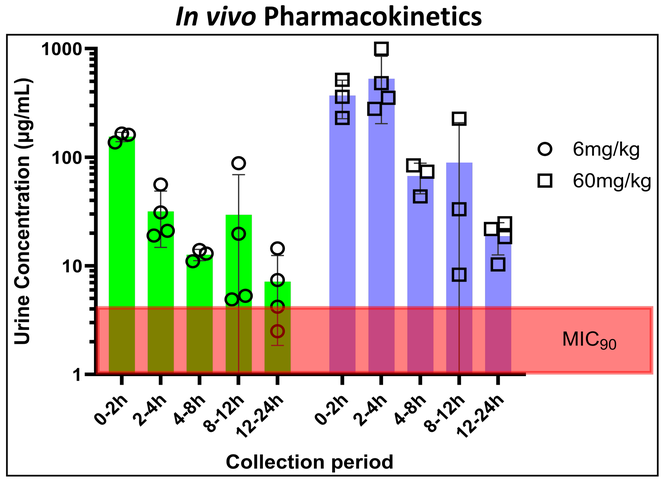
Strain		Sequencing Results		MIC (µg/mL)							
		LpxC	FabZ	FG-960	Fold-Change (vs WT)	CIP[a]	Fold-Change (vs WT)	TOB[b]	Fold-Change (vs WT)	MPM[c]	Fold-Change (vs WT)
E. coli 25922		WT	WT	0.25	NA	0.016	NA	0.5	NA	0.016	NA
E. coli ATCC 25922 clones from FG-960 FoR[d] plates	#1	WT	A71S	2	8	0.008	2	0.5	1	0.016	1
	#2	WT	A71V	2	8	0.008	2	0.25	0.5	0.008	0.5
	#3	WT	K102N	4	16	0.008	2	0.5	1	0.016	1
	#4	WT	R100S	8	32	0.008	2	0.125	0.25	≤0.008	≤0.5
	#5	WT	C139Y	2	8	0.008	2	0.5	1	0.016	1
	#6	WT	P22L	2	8	0.008	2	0.25	0.5	≤0.008	≤0.5
	#7	WT	G129E	2	8	0.008	2	0.125	0.25	≤0.008	≤0.5
	#8	WT	A71S	4	16	0.016	1	0.5	1	0.016	1

[a] – ciprofloxacin; [b] – tobramycin; [c] – meropenem; [d] – FoR, frequency of resistance

TOP: Spontaneous frequency of resistance (FoR) results for FG-960, two comparator LpxC inhibitors, and ciprofloxacin evaluated against *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 13883.

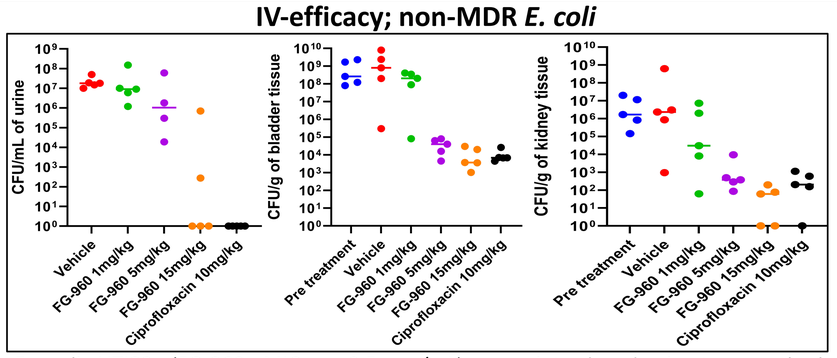
BOTTOM: Sequencing and antibiogram phenotyping of select *E. coli* ATCC 25922 spontaneous-resistant mutants recovered after FG-960 challenge in FoR studies.

In vivo Pharmacokinetics



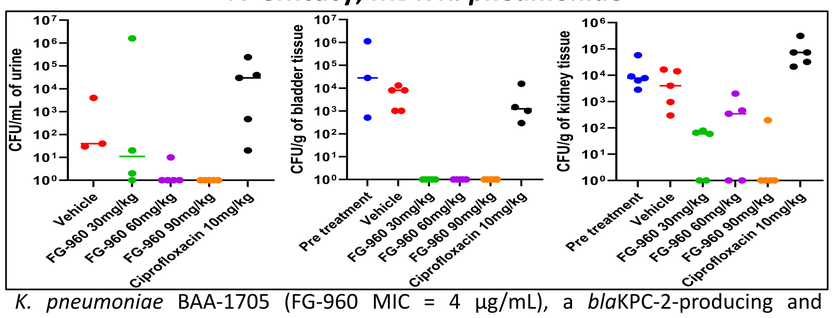
Kinetic recovery of intact FG-960 from Sprague Dawley rat urine (n=4) after IV administration of 6 mg/kg (green bars, circles) and 60 mg/kg (blue bars, squares) doses. Urine was recovered via metabolic cage collection. For reference, the anticipated MIC_{90} for all target uropathogens is shaded in red.

IV-efficacy; non-MDR *E. coli*



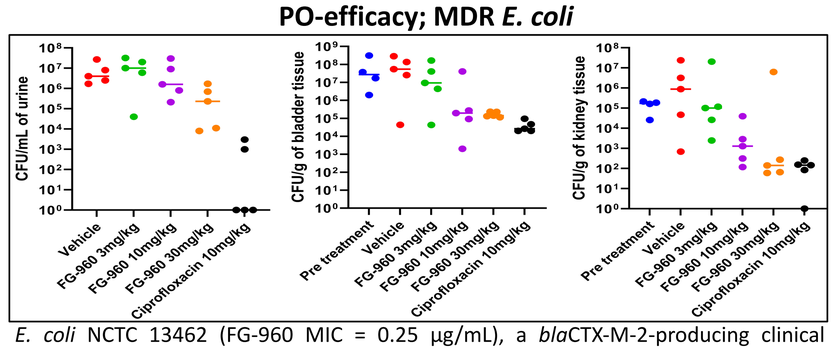
E. coli UTI89 (FG-960 MIC = 0.5 µg/mL) was introduced via transurethral administration 24 hours prior to initiation of therapy. FG-960 was administered BID for 72 hours prior to bioburden recoveries from the urine (left), bladder (middle), or kidney (right). Ciprofloxacin was included as a comparator.

IV-efficacy; MDR *K. pneumoniae*



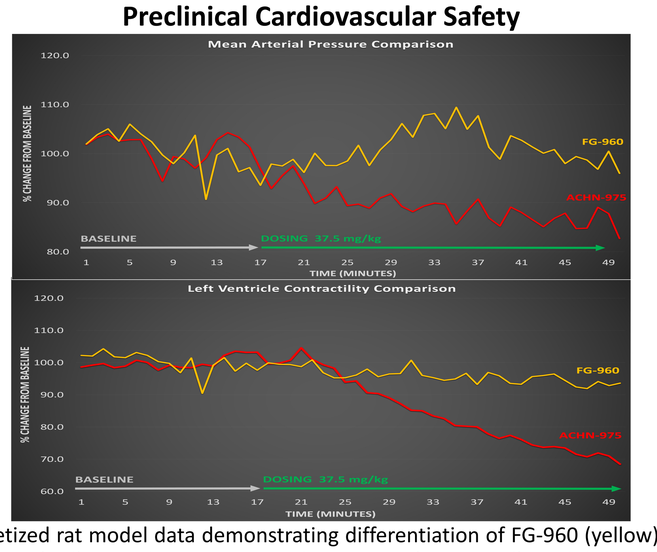
K. pneumoniae BAA-1705 (FG-960 MIC = 4 µg/mL), a *bla*KPC-2-producing and fluoroquinolone-resistant clinical isolate, was introduced via transurethral administration 24 hours prior to initiation of therapy. FG-960 was administered QID for 72 hours prior to bioburden recoveries from the urine (left), bladder (middle), or kidney (right). Ciprofloxacin was included as a comparator.

PO-efficacy; MDR *E. coli*



E. coli NCTC 13462 (FG-960 MIC = 0.25 µg/mL), a *bla*CTX-M-2-producing clinical isolate, was introduced via transurethral administration 24 hours prior to initiation of therapy. FG-960 was administered BID for 72 hours prior to bioburden recoveries from the urine (left), bladder (middle), or kidney (right). Ciprofloxacin was included as a comparator.

Preclinical Cardiovascular Safety



Anesthetized rat model data demonstrating differentiation of FG-960 (yellow) from ACHN-975 (red). Both mean arterial pressure (top panel) and left ventricle contractility (bottom panel) were reduced both preclinically and in a Phase 1 clinical trial by ACHN-975, but no significant changes in either parameter were observed when FG-960 was administered at 37.5 mg/kg.

Acknowledgements

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