



Blacksmith Corporate Overview

*Developing medicines targeting metal-dependent enzymes with a focus on
Oncology, DNA Damage Response, and Infectious Disease*

Needham Healthcare Conference
April 2024



Blacksmith Medicines: Executive summary

Mission:

To be the leading biopharma dedicated to discovering and developing medicines targeting metalloenzymes



Purpose-built platform for metalloenzyme-targeted medicines



Robust internal pipeline focused on oncology and infectious disease with lead program in IND-enabling



Multiple drug discovery collaborations including Roche, Eli Lilly, and Zoetis



Investors include Eli Lilly, Evotec, MP Healthcare Partners, MagnaSci Ventures, and Alexandria Ventures

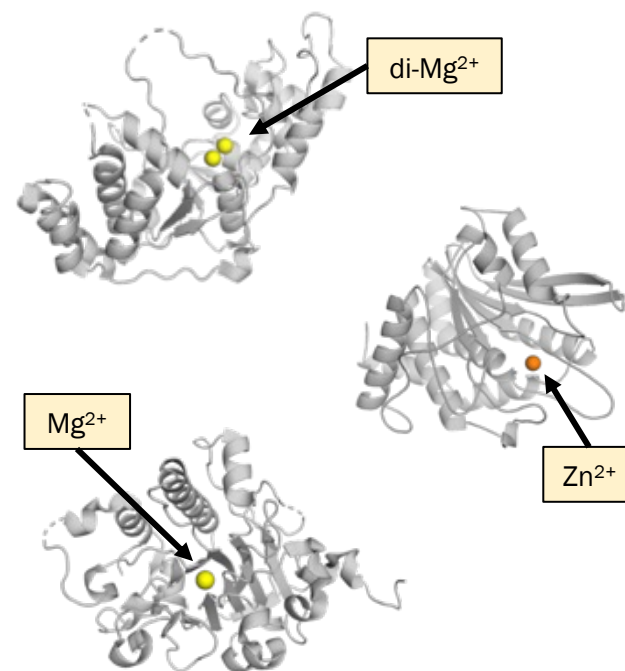
Introduction: Metalloenzymes & metalloenzyme inhibitors

What are metalloenzymes?

Metalloenzymes are a group of enzymes that bind a metal ion as a cofactor and utilize it directly to catalyze critical biochemical reactions

Over 30% of known enzymes (more than 1,000 in total) are metalloenzymes, covering all major enzyme classes and most biological processes

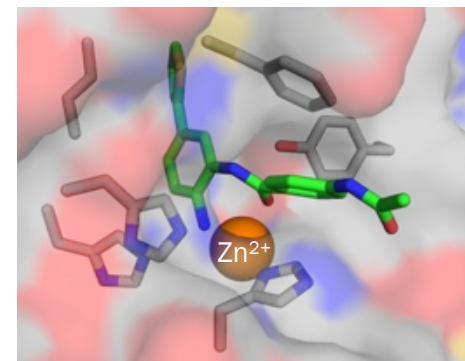
Metal ions like magnesium, zinc, iron, manganese, and copper are the essential ingredient in metalloenzymes



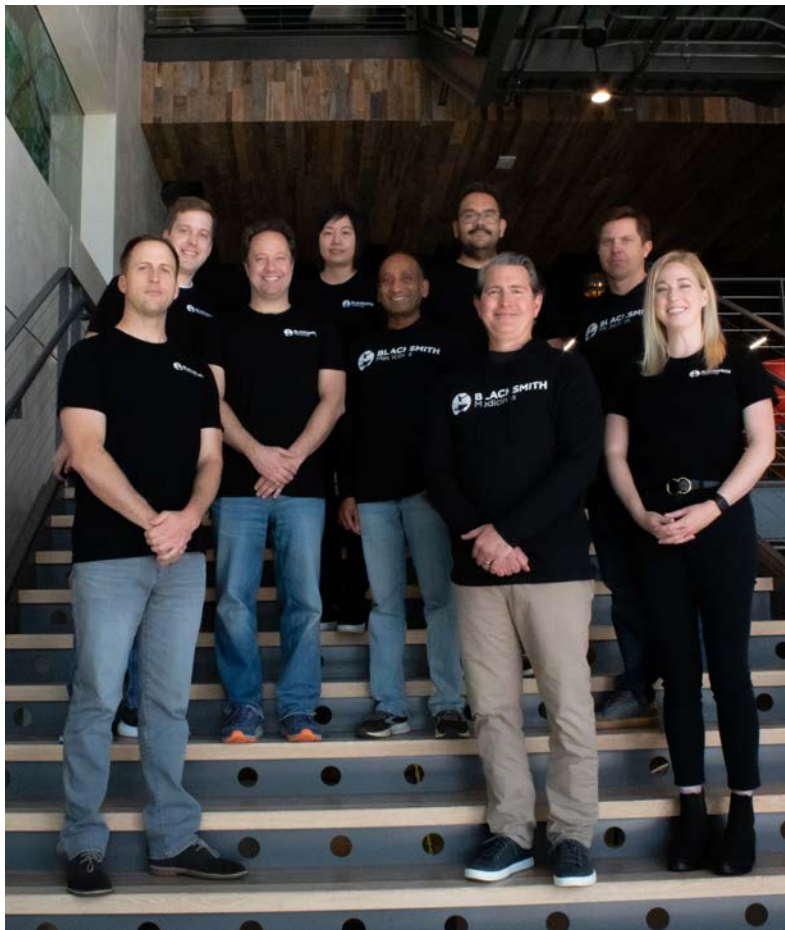
What is a metalloenzyme inhibitor?

Metalloenzyme inhibitors are small molecules designed to prevent catalysis by binding to the protein-bound metal cofactor

The BLACKSMITH inhibitor design process starts with proprietary metal binding pharmacophores (MBPs), which are then elaborated to increase potency, selectivity, and other drug-like properties



Blacksmith Medicines: Who we are and what we do



Blacksmith Medicines (fka Forge Therapeutics) launched in 2015

- Originally developed by our scientific co-founder Prof. Seth Cohen at Univ. of California San Diego (UCSD)
- Unique team of **bio-inorganic chemists** paired with experienced medicinal chemistry, cell & molecular biology, biochemistry, and structural biology

Focus on targets of significant unmet need & pharma interest

- Targets with validated biology but chemistry limitations that we can solve
- Unique ‘problem-solution’ approach using fragment-based & structure-based drug design leveraging in-house computational modeling

Problem: Pharma has struggled to develop metalloenzyme inhibitors

Pharma's Chemistry Problem

We have a 'hot' list of metalloenzyme targets but no chemistry tools...until now with our Blacksmith collaboration



Pharma's libraries do not contain these types of metal binding fragments; we use the same pharmacophores that everybody else uses and when we change an atom to optimize or get around IP concerns we lose potency



Everyone has hydroxamic acid nightmare stories...lack of selectivity, poor PK, and toxicity...ultimately these programs get shelved, not because of biology, but a lack of chemistry

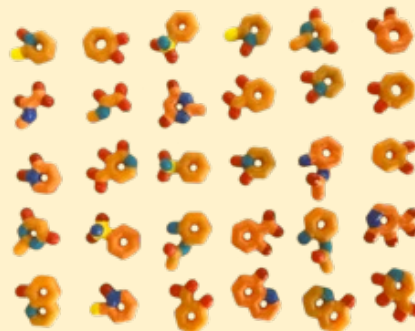


Upon hindsight, it would have been smart to have bioinorganic chemists on our team since we had multiple metalloenzyme projects...that would have been a good idea



Blacksmith's Solution

Rationally designed metal-binding fragments:
unique and diverse 'starting points'



Key differentiation: Focused library of >1500 MBPs with various donor atom configurations and metal-binding characteristics (e.g. donor geometry, pK_a)

- *Speed*: MBPs bound to active site metal 'jump start' design of full-length inhibitors
- *Options*: Multiple novel scaffolds, tunable drug-like properties, and free & clear IP

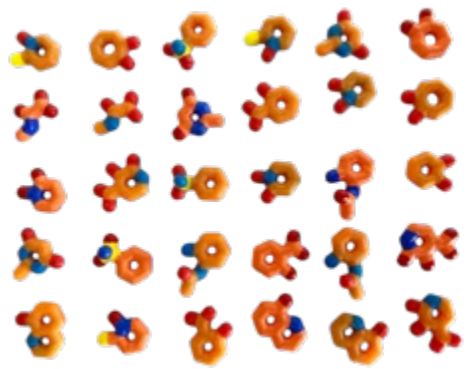
Solution: The power of fragments & key bioinorganic know-how to accelerate lead identification

Step 1: Establish biochemical assay



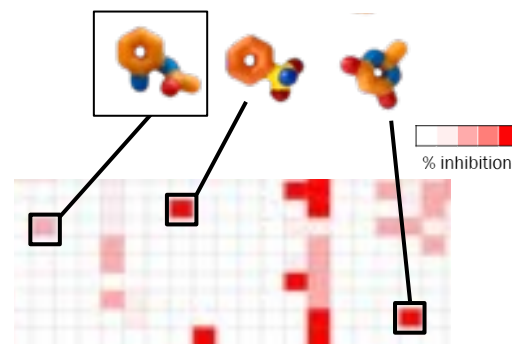
Metalloenzyme target

Step 2: Screen MBP library



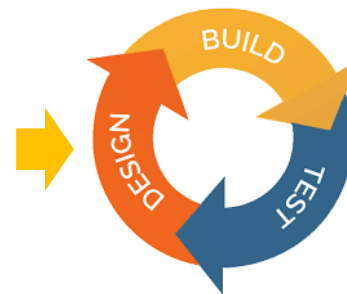
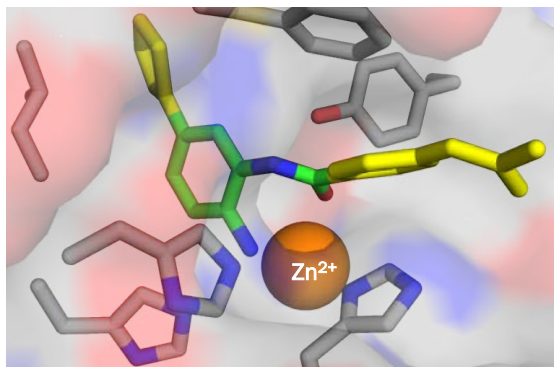
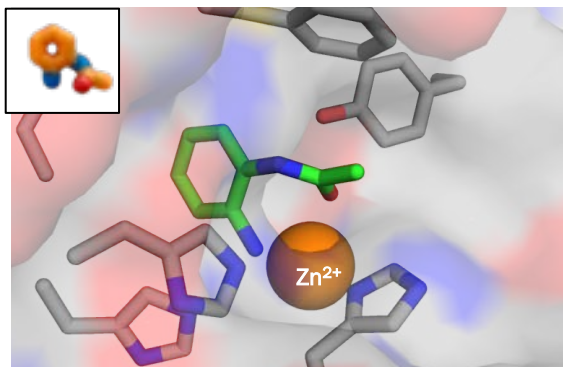
MBP fragment library

Step 3: Identify unique & selective MBP 'hits'



MBP inhibition heat map

Step 4: Apply key bioinorganic know-how to model 'hits' with proper location & orientation followed by fragment growth strategy using Blacksmith's proprietary computational process



Lead ID

Pipeline: Novel oncology & infectious disease medicines supported by multiple pharma partners & federal government


Therapeutic areas	Target	Discovery	Lead optimization	IND enabling	Phase 1	Partner
Infectious disease	LpxC Zn ²⁺ deacetylase	MDR urinary tract infections		✓		NIH* \$17.2M contract thru Phase 1
	LpxC Zn ²⁺ deacetylase	MDR lung infections	✓			Roche \$190M total deal plus royalties
	LpxC Zn ²⁺ deacetylase	MDR sexually-transmitted infections				
Oncology	FEN1 di-Mg ²⁺ nuclease	DNA replication & repair				
	QPCTL Zn ²⁺ transferase	Solid tumor immunotherapy				
	Undisclosed	Novel synthetic lethal pairings				
Collaborations	Undisclosed	Multi-TAs	✓			Lilly \$300M total deal plus royalties
	Undisclosed	Animal health	✓			zoetis Economics not disclosed

✓ Externally funded

Target strategy: Important targets that have been challenging to drug due to chemistry limitations that we can fix with our platform

Partnering strategy: Partnerships are key to our success providing funding, investment, access to technology, and external validation

*This project has been funded in whole or in part 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. 75N93022C00060

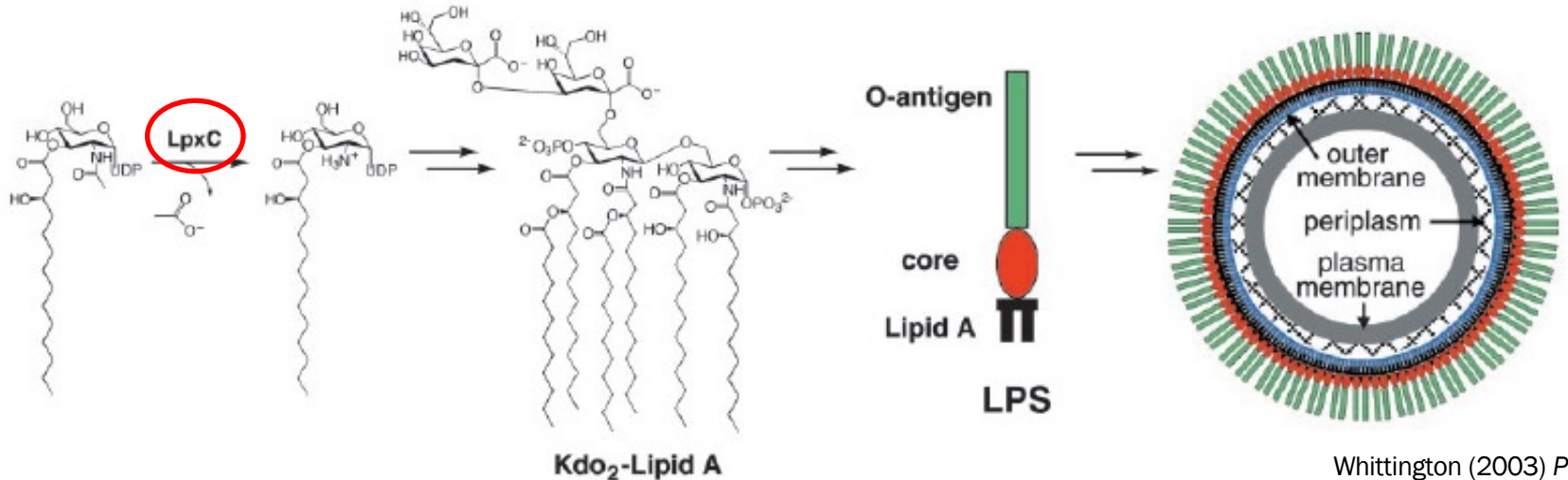


**LpxC: Highly valuable
antibiotic target plagued
by chemistry limitations**



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LpxC as a novel antibacterial target: Essential for biosynthesis of Gram-negative bacterial outer membrane

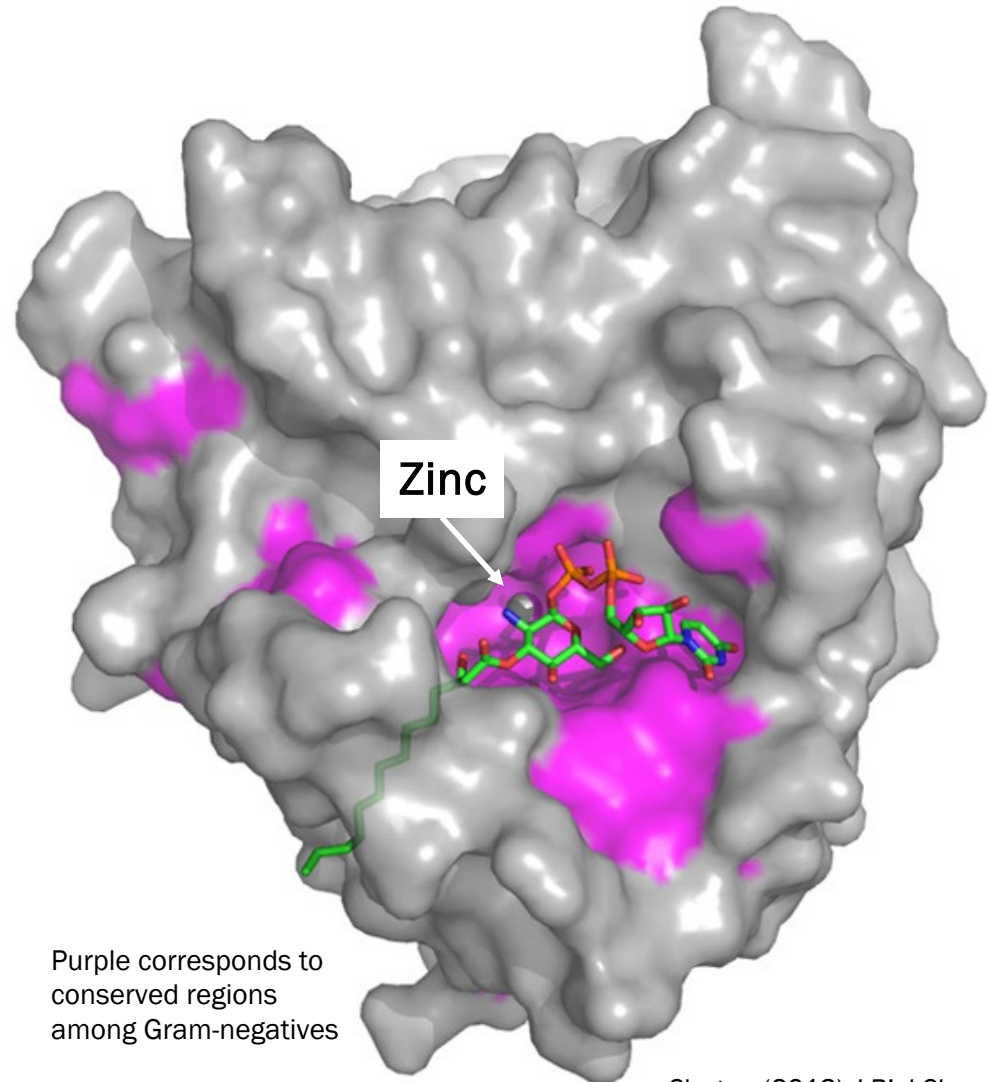


Whittington (2003) PNAS

- LpxC responsible for synthesis of lipid A (anchor of outer membrane) in Gram-negative bacteria
 - Removes acetyl group from UDP-GlcNAc to produce UDP-GlcN which is converted to lipid A
- Not found in humans; essential for Gram-negative bacterial viability

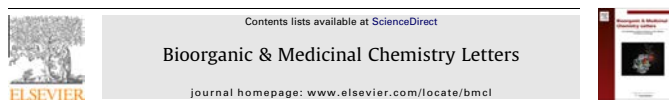
LpxC drug discovery: Well-understood biology, assays in place, plus structural data

- LpxC catalytic site contains single bound zinc (silver sphere), hydrophobic tunnel, and UDP pocket
- LpxC inhibitors are designed to mimic three critical substrate interactions in LpxC active site:
 1. *Metal binding pharmacophore (MBP) to anchor to zinc*
 2. *Long tail to fit in tunnel*
 3. *Interact with key lysine in UDP pocket*



Clayton (2013) *J Biol Chem*

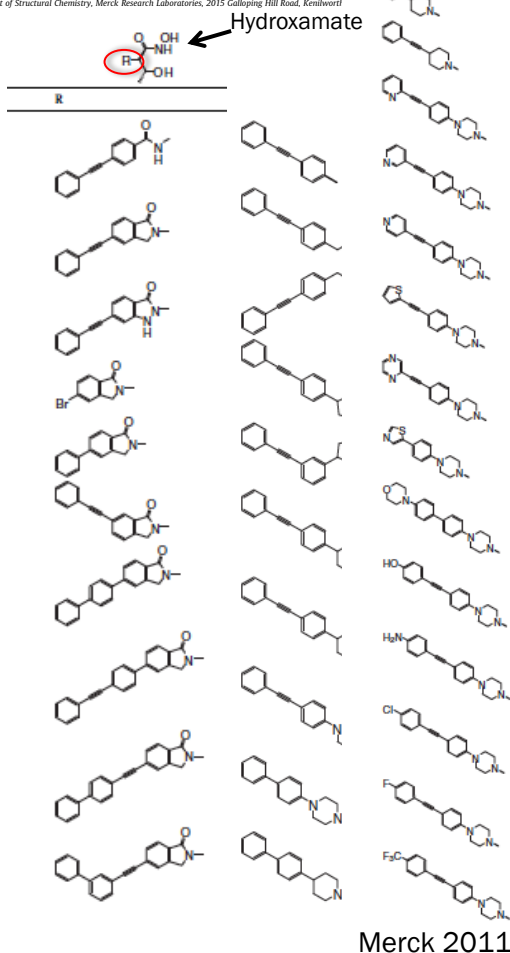
Problem: Previous attempts have optimized interactions with the tunnel & pocket but limited success with metal-binding



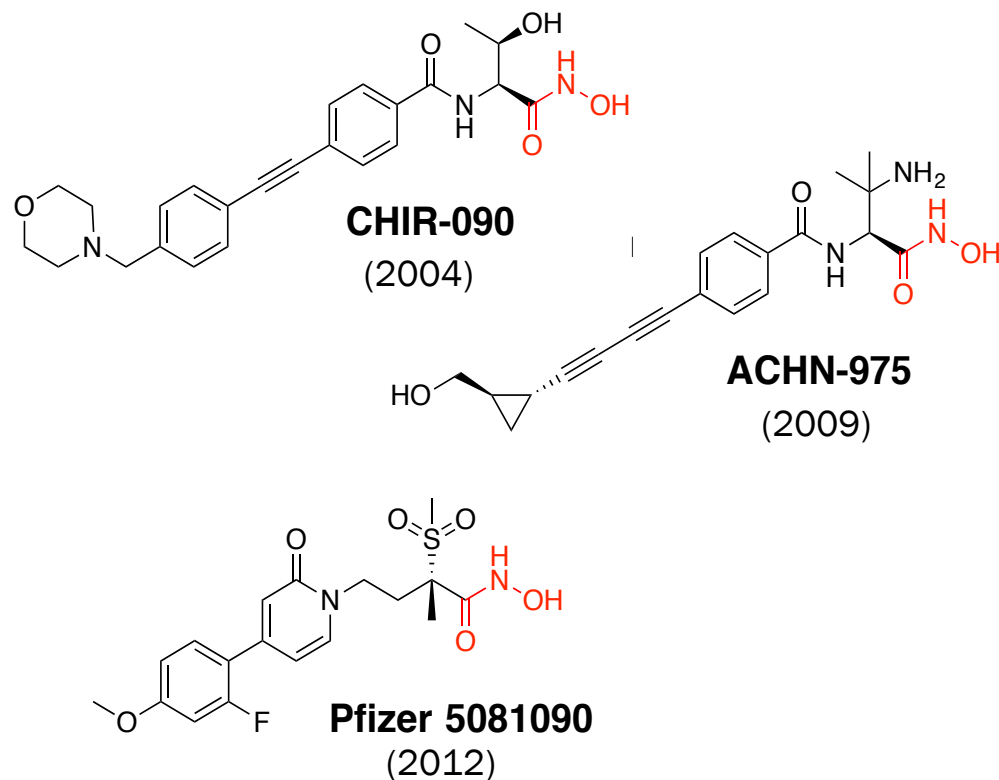
Design and synthesis of potent Gram-negative specific LpxC inhibitors

U. Faruk Mansoor^{a,*}, Dilrukshi Vitharana^a, Panduranga Adulla Reddy^a, Dayna L. Daubaras^b, Paul McNicholas^b, Peter Orth^c, Todd Black^b, M. Arshad Siddiqui^a

^aDepartment of Chemistry, Merck Research Laboratories, 320 Bent Street, Cambridge, MA 02141, USA
^bDepartment of Biology, Merck Research Laboratories, 2015 Gallop Hill Road, Kenilworth, NJ 07033, U.S.A.
^cDepartment of Structural Chemistry, Merck Research Laboratories, 2015 Gallop Hill Road, Kenilworth, NJ 07033, U.S.A.

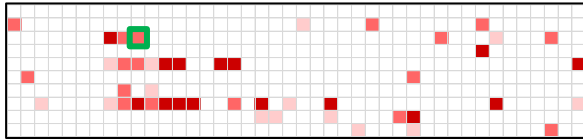


- Previous LpxC inhibitors relied on **hydroxamate** chemistry as MBP (in **RED**)
- Potent *in vitro*
- Suffer from metabolism, high clearance *in vivo* and cardiovascular toxicity
- **No** approved drugs



Solution: Blacksmith platform provides alternatives to hydroxamate and has discovered novel LpxC inhibitors

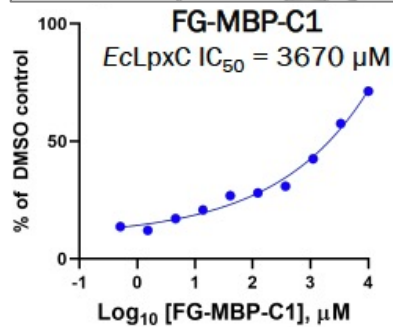
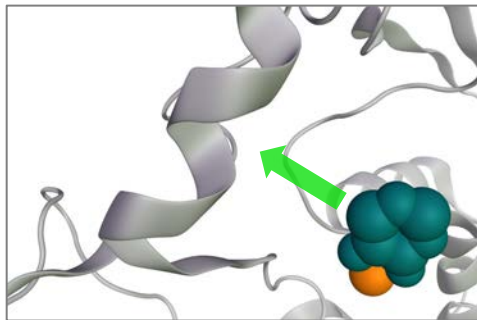
1 MBP screen identifies new starting points



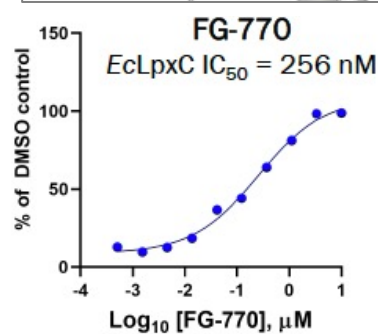
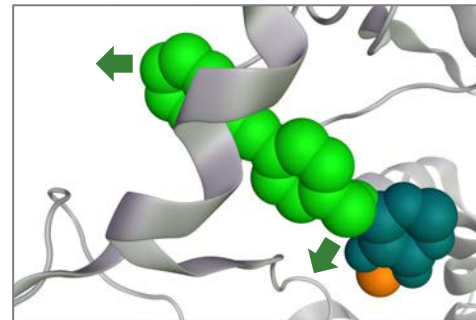
Program activities/achievements:

- ✓ Established enzyme assay
- ✓ Screened MBP library
- ✓ Verified MBP dose response
- ✓ Established structural biology
- ✓ Designed & synthesized full-length inhibitors
- ✓ Demonstrated *in vitro* activity w/MDR strains
- ✓ De-risked resistance liabilities
- ✓ Demonstrated *in vivo* efficacy and safety

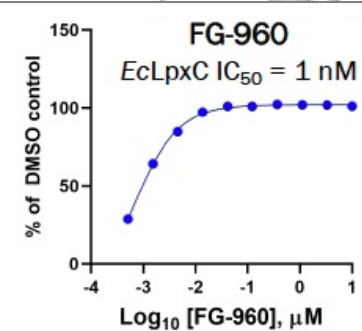
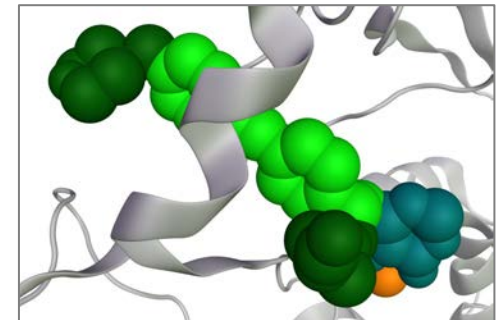
2 Bioinorganic modeling of MBPs to develop *metal binding hypothesis*



3 Elaborate MBPs/initiate computational to test



4 Chemistry campaign to build desired potency/properties

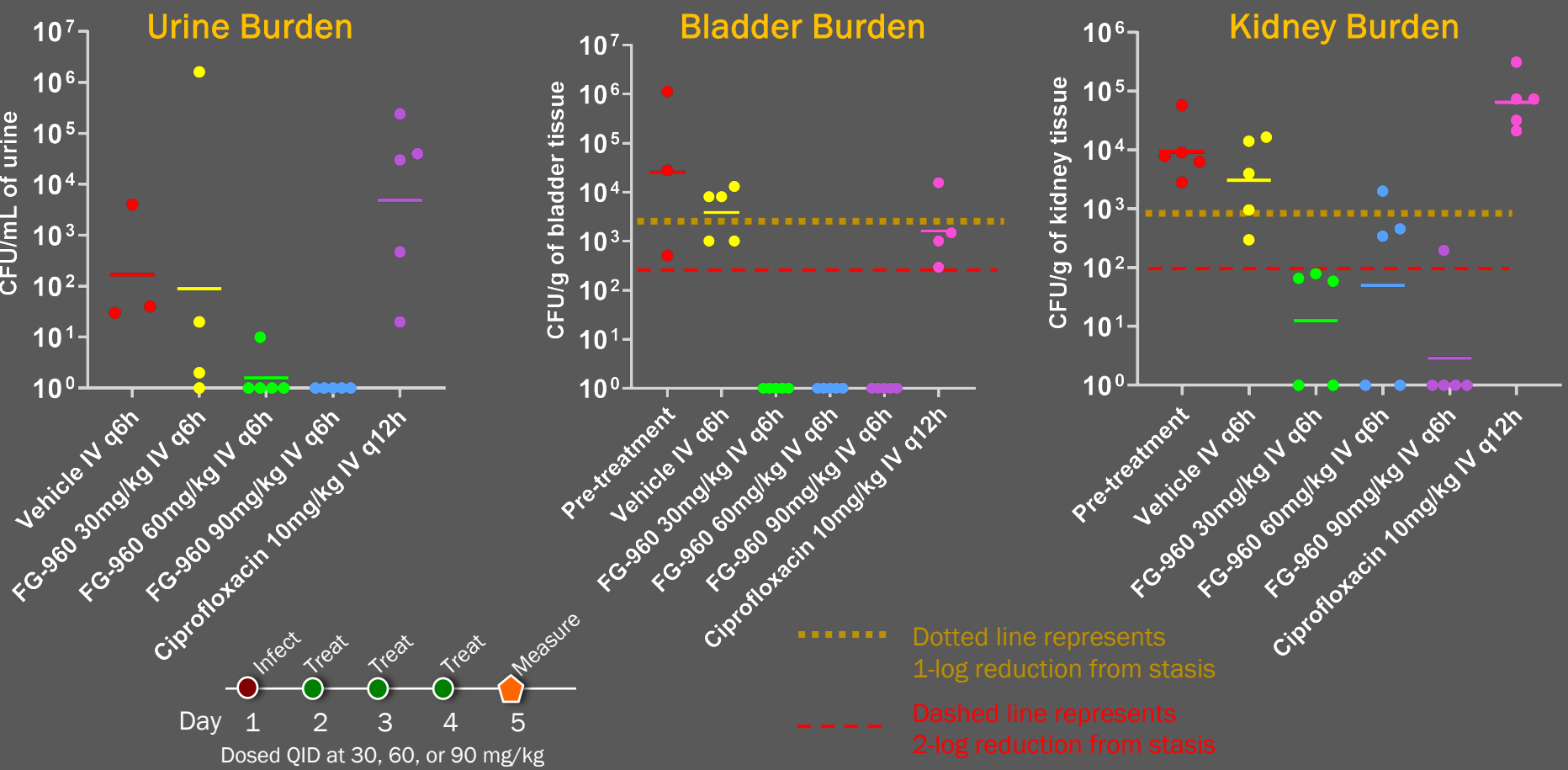


In vitro data: Potent Gram-negative bacteria killing

Species (# strain)	MIC ₉₀ (µg/ml)			
	FG-960	CAZ	NIT	GEN
<i>E. coli</i> (208)	1	>16	32	>16
<i>K. pneumoniae</i> (207)	4	>16	>128	>16
<i>E. cloacae</i> (133)	4	>32	128	4
<i>P. mirabilis</i> (96)	4	0.5	128	16

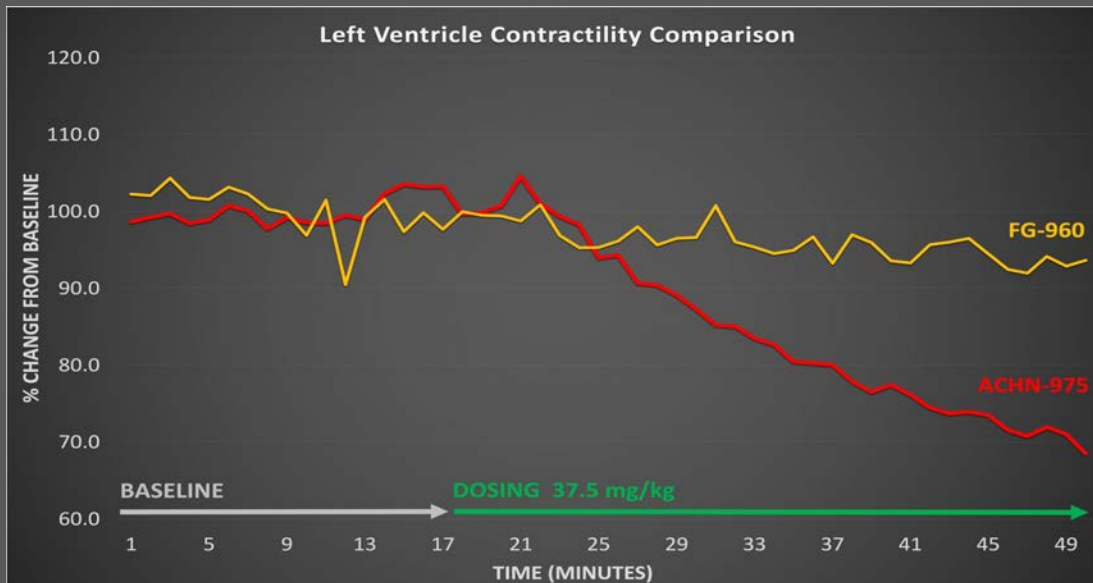
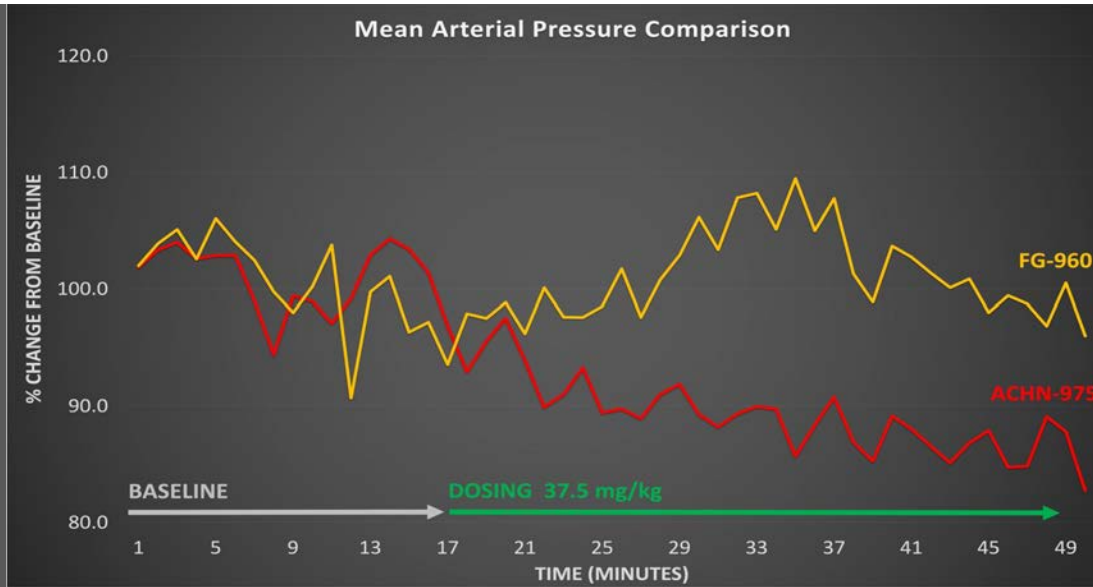
FG-960: Novel non-hydroxamate LpxC inhibitor able to kill drug-resistant Gram-negative bacteria

In vivo data: Efficacy against drug resistant *K. pneumoniae*



- Rapid dose-dependent bacteria killing in UTI model (urine, bladder and kidney)
- Gram-negative drug resistant strain: *K. pneumoniae* ATCC BAA-1705 (*bla_{KPC}*) is fluoroquinolone- and carbapenem-resistant


Differentiation from legacy LpxC inhibitors: FG-960 does not show CV effects induced by ACHN-975



Differentiation: FG-960 does not induce hemodynamic effects in rats.

Both compounds dosed at 75 mg/kg IV

Status & next steps: IND-enabling followed by FIH studies

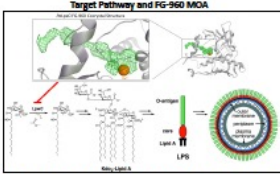
Advanced preclinical *in vitro* and *in vivo* characterization of a novel, non-hydroxamate-based LpxC inhibitor for the intravenous and oral treatment of multidrug-resistant Enterobacteriales 

J. Munguia, K. Taganan, S. Agarwala, D. Martin, J. Fan, D. Lonergan, D. Puerta, Z. Zimmerman, and A. Tomaras
BlackSmith Medicines, Inc. San Diego, CA, USA

Abstract
LpxC is an essential metalloenzyme that Gram-negative bacteria require for outer membrane biogenesis. Previous antibacterial efforts have focused on hydroxamate-based small molecules to engage LpxC's catalytic site. 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 Enterobacteriales, and is currently being advanced as a treatment for urinary tract infections (UTIs).

FG-960 is highly-potent against the LpxC enzyme (IC₅₀ < 1 nM), and has strong whole cell activity against panels of contemporary MDR and non-MDR Enterobacteriales isolates (MIC₅₀ = 4 µg/mL; n= 973 strains). Static time kill studies demonstrate FG-960's novel bactericidal activity, with regrowth suppression evident at concentrations >2X MIC. When evaluated in extended frequency of resistance assay, FG-960 shows spontaneous resistance emergence at frequencies consistent with historical LpxC inhibitors (10⁻¹⁰-10⁻¹²) at 4X MIC against multiple MDR and non-MDR strains. Pharmacokinetic studies have demonstrated dose-proportional exposure 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 0.50 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

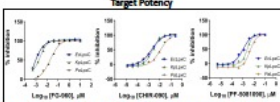


Role of LpxC in Gram-negative bacterial outer membrane biogenesis (adapted from Whittam et al, PNAS July 2002), and the inhibitory mechanism of action for FG-960.

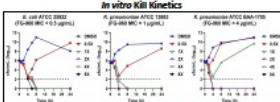
Frequency and Mechanism of FG-960 Resistance

Compound	Parent MIC	6 coil ATCC 25922	6 penicillin ATCC 25922
FG-960	4	4	4
AC268-075	4	4	4
FG-960-1000	4	4	4
Clarithromycin	4	4	4

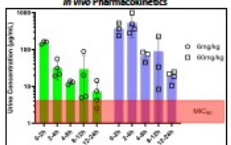
Target Potency



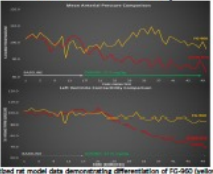
***In vitro* Kill Kinetics**



***In vivo* Pharmacokinetics**



Preclinical Cardiovascular Safety




ACKNOWLEDGEMENTS
We are grateful to Swarna Vaidi and David Corbett (Invitac), as well as to Karen Shaw, Lynn Silver, Mike Barbachano, Seth Cohen, Lloyd Payne, John Rao, Peter Wertz, and Mark Whitaker (BlackSmith SAS) for their experimental and technical support. We also thank CAP-X for prior financial support. This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institute of Health, Department of Health and Human Services, under Contract No. 75N6302200060.

Status and next steps

- Novel non-hydroxamate LpxC inhibitor single digit nanomolar potency
- *In vivo* efficacy in multiple infection models using IV and oral routes of administration
- Completed IND-enabling toxicology with no concerns
- Currently undergoing scale-up for clinical trials
- Fully-funded through the end of Phase 1 (IV and oral) by \$17.2M contract with NIH/NIAID

See recent poster from Gordon Research Conference (March 2024) at www.BlackSmithMedicines.com



**FEN1: Novel DNA Damage
Repair target with several
synthetic lethal
relationships**



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FEN1, a novel oncology target: DNA replication and repair

FEN1

Enzyme class: Nuclease

Catalytic metal: $Mg^{2+}Mg^{2+}$



Mechanism

- FEN1 cleaves 5' single stranded DNA flaps during lagging strand DNA replication (Okazaki fragment maturation) or DNA damage repair
- The divalent Mg^{2+} metal ion in the FEN1 enzyme active site is responsible for its activity

Biology

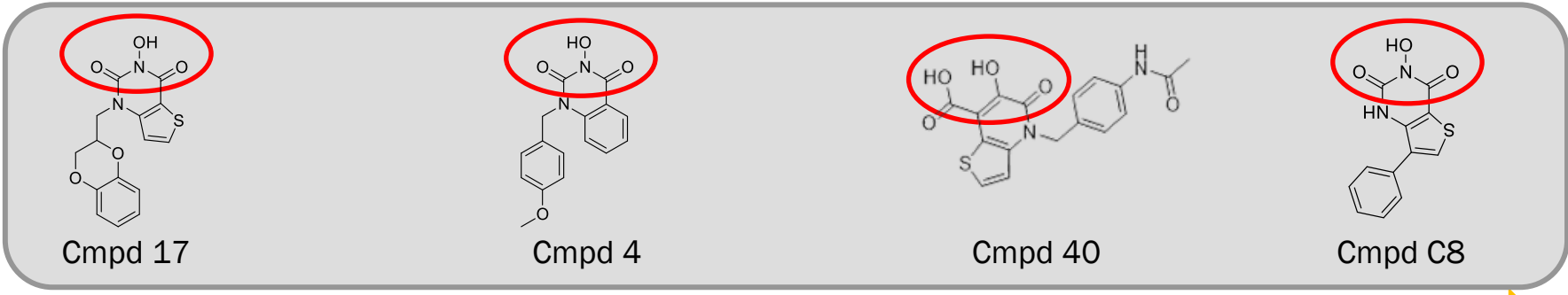
- FEN1 is highly conserved from yeast to humans indicating its important to cell biology
- FEN1 is highly expressed in proliferative tissue and even higher in cancer

Therapeutic relevance

- Targeting FEN1 has been proposed to be an important therapeutic strategy alone or synergistic with current treatments, however, chemistry limitations have hampered progress

Problem: Previous FEN1i had limited metal-binding pharmacophore chemistry, selectivity, and cell potency

Mg²⁺ binding chemistry



2005
Tumey *et al.*
Athersys

2016
Exell *et al.*
Astra Zeneca

2019
Ideaya
Biosciences

2020
Guo *et al.*
UC San Diego

The identification and optimization of a N-hydroxy urea series of flap endonuclease 1 inhibitors

L. Nathan Tumey, David Bom, Bayard Huck, Elizabeth Gleason, Jianmin Wang, Daniel Silver, Kurt Brunden, Shery Boozer, Stephen Rundlett, Bruce Sherf, Steven Murphy, Tom Dent, Christina Leventhal, Andrew Bailey, John Harrington and Yousef L. Benjami

Athersys, Inc., 2200 Carnegie Ave., Cleveland, OH 44115, USA
Received 30 July 2004; revised 20 October 2004; accepted 30 October 2004
Available online 25 November 2004

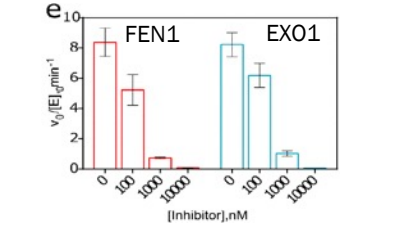
United States Patent
Tumey *et al.* (19) Patent No.: **US 7,947,691 B2**
(41) Date of Patent: **May 24, 2011**

(54) **CYCLO-N-HYDROXY UREA DERIVATIVES AS INHIBITORS OF FLAP ENDONUCLEASE AND USES THEREOF**
(57) **Inventors:** Lawrence N. Tumey, New Windsor, NY (US); Vincent Bennett, Oyster Heights, OH (US); Bayard Huck, University Heights, OH (US); David C. Bom, Broadview Heights, OH (US)
(73) **Assignee:** Athersys, Inc., Cleveland, OH (US)

Cellularly active N-hydroxyurea FEN1 inhibitors block substrate entry to the active site

Jack C Exell, Mark J Thompson, L David Finger, Steven J Shaw, Judit Debreczeni, Thomas A Ward, Claire McWhirter, Cathrine L B Söberg, Daniel Martínez Molina, W Mark Abbott, Clifford D Jones, J Willem M Nissink, Stephen T Durant & Jane A Grasty

Nature Chemical Biology 12, 815–821(2016) | [Cite this article](#)



N-hydroxy urea inhibitors are **not selective** for FEN1

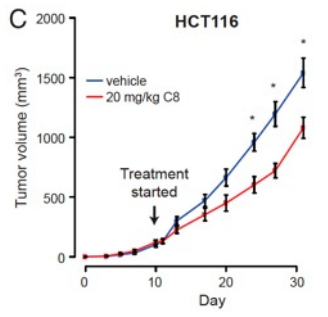
(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)
(18) World Intellectual Property Organization
International Bureau
(43) International Publication Date: **04 April 2019 (04.04.2019)**
W I P O | P C T
(10) International Publication Number: **WO 2019/067442 A1**

(51) International Patent Classification: A61K 31/443 (2006.01)
(52) International Application Number: PCT/US2018/02540
(53) International Filing Date: 25 September 2018 (25.09.2018)
(55) Filing Language: English
(56) Publication Language: English
(57) Priority Date: 25 September 2017 (25.09.2017) US 15,910,824
(71) Applicant: IDEAYA BIOSCIENCES, INC. (US); 7000 Shattuck Court, Suite 300, San Francisco, California 94120 (US)

Example #	FEN1 IC ₅₀ (uM)	EXO1 IC ₅₀ (uM)	XPG IC ₅₀ (uM)
39	*	*	*
40	***	*	*
41	***	*	*

*** IC₅₀ < 0.25 uM, ** 0.25 < IC₅₀ < 0.75 uM, * 0.75 < IC₅₀ < 2.25 uM, * IC₅₀ 2.25 < IC₅₀ < 10 uM, - IC₅₀ > 10 uM

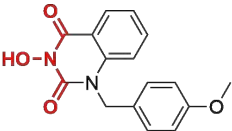
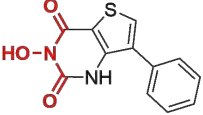
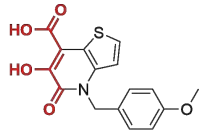
FEN1 **selectivity** observed but **limited cell activity**



C8 reduces tumor growth *in vivo* (despite poor potency and PK)

Athersys N-hydroxy urea patent issued in 2011

Solution: Potent & selective lead scaffold with novel MBP

Company	Structure	ID	FEN1 IC ₅₀ (μM)	EXO1 IC ₅₀ (μM)	FEN1 vs. EXO1 selectivity (fold)
Astra Zeneca		Cmpd 4	0.21	0.72	3.4
Athersys		Cmpd 8	0.13	0.41	3.2
Ideaya		Cmpd 12	2.7	14	5.2
Blacksmith Medicines	Compound utilizing a novel metal-binding pharmacophore	BSM-1516	0.007	0.46	65

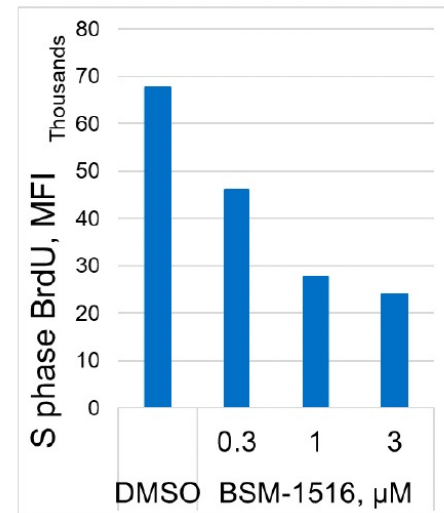
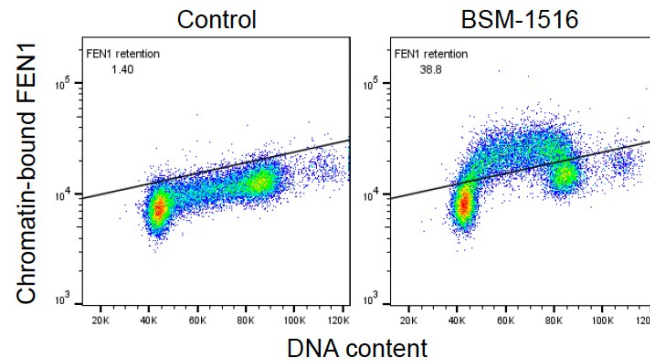
BSM-1516: To our knowledge, the most potent & selective FEN1 inhibitor to date PLUS contains a novel MBPs and does not utilize an N-hydroxy urea or carboxylic acid

BSM-FEN1: Cellular target engagement and mechanism

FEN1 target engagement by cellular thermal shift assay

Company	ID	CETSA EC_{50} (μ M)
Astra Zeneca	Cmpd 4	1.2
Athersys	Cmpd 8	0.21
Ideaya	Cmpd 12	>100
Blacksmith Medicines	BSM-1516	0.024

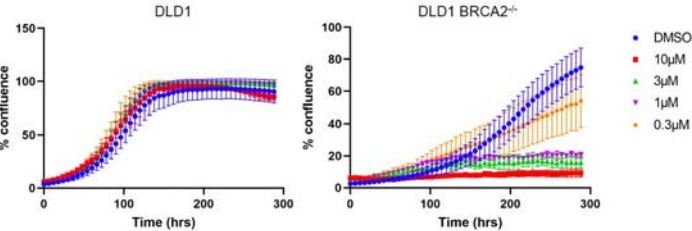
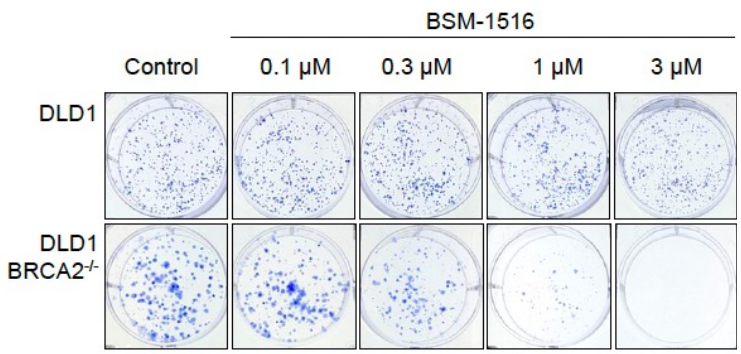
FEN1 protein bound to chromatin in cells treated with BSM-1516 and impaired S-phase progression



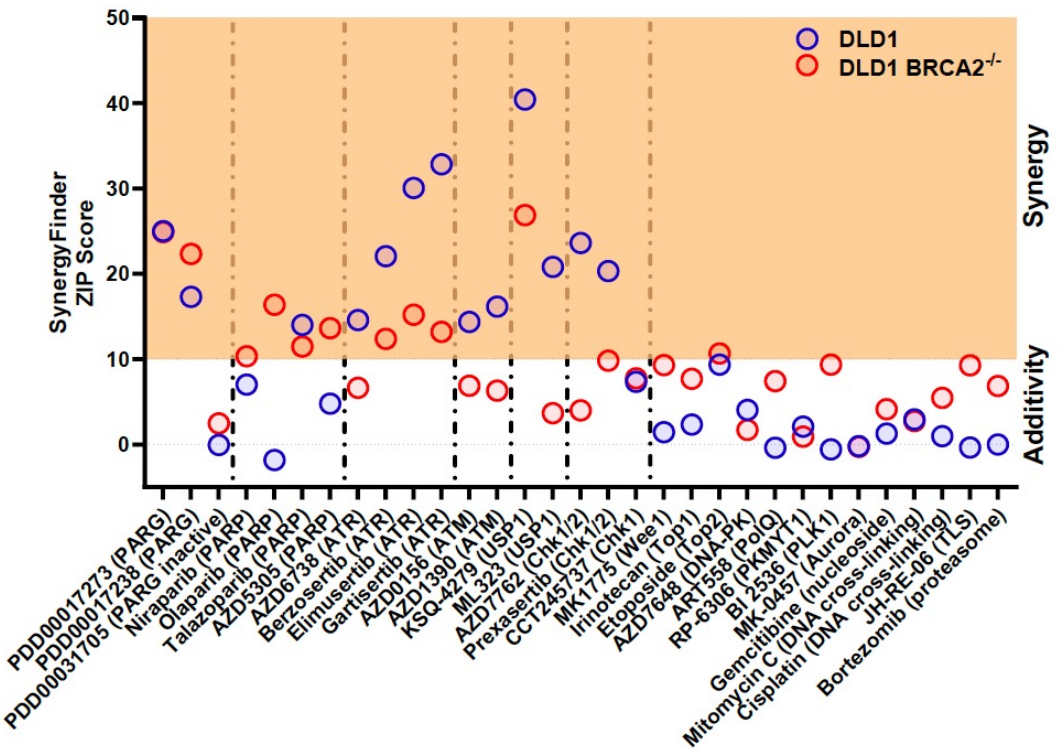
BSM-1516 stabilizes FEN1 and increases its chromatin retention in S phase that is accompanied by slowdown of DNA replication

BSM-FEN1: Synthetic lethal with defects in HRR and synergizes with inhibitors of USP1, PARP, PARG, ATR and Chk1

BRCA2-deficient cells are more sensitive to BSM-1516 (clonogenic survival and cell proliferation assays)



BSM-1516 shows strong synergy with multiple DDR drug classes on the market and in the clinic including inhibitors of PARP, PARG, USP1 and ATR




BRCA2 gene deficiency causes sensitivity to FEN1 inhibition and BSM-1516 synergizes with several DNA damage inhibitors

Status & next steps: Lead optimization & in vivo

7148 **Small molecule inhibitor of FEN1 nuclease utilizing a novel metal-binding pharmacophore synergizes with inhibitors of USP1, PARP, PARG and ATR**

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Abstract

The endonuclease FEN1 is a critical protein in DNA replication and repair. FEN1 is an attractive target for development of anticancer therapies as its overexpression in many tumor types and its role in DNA replication and repair make it a potential target for cancer therapy. FEN1 is a member of the FEN1 family of nucleases and is involved in DNA replication and repair. FEN1 is a member of the FEN1 family of nucleases and is involved in DNA replication and repair. FEN1 is a member of the FEN1 family of nucleases and is involved in DNA replication and repair.

Cellular Target Engagement and Mechanism of Action

BSM-1516 binds to FEN1 and blocks its activity. FEN1 is a member of the FEN1 family of nucleases and is involved in DNA replication and repair. FEN1 is a member of the FEN1 family of nucleases and is involved in DNA replication and repair. FEN1 is a member of the FEN1 family of nucleases and is involved in DNA replication and repair.

Viability assays: ERCC2-deficient cells are >10 fold more sensitive to FEN1 inhibitor than isogenic wild-type control

ERCC2-deficient cells are more sensitive to FEN1 inhibitor than isogenic wild-type control. ERCC2-deficient cells are more sensitive to FEN1 inhibitor than isogenic wild-type control. ERCC2-deficient cells are more sensitive to FEN1 inhibitor than isogenic wild-type control.

Drug combination data: BSM-1516 synergizes with inhibitors of USP1, PARP, PARG, ATR and Chk1

BSM-1516 synergizes with inhibitors of USP1, PARP, PARG, ATR and Chk1. BSM-1516 synergizes with inhibitors of USP1, PARP, PARG, ATR and Chk1. BSM-1516 synergizes with inhibitors of USP1, PARP, PARG, ATR and Chk1.

Biochemical assays: Improved potency and greater selectivity of BSM-1516 compared to historic FEN1 inhibitors

Compound	IC50 (nM)	IC90 (nM)	IC95 (nM)	IC99 (nM)
BSM-1516	0.21	0.25	0.34	0.44
BSM-1516	0.10	0.41	0.52	0.62
BSM-1516	0.27	0.48	0.55	0.65

Summary

- Blacksmith Medicines PRD approach for multi-targeted inhibition of cell cycle proteins.
- Pharmacologic inhibition of FEN1 in combination with inhibitors of USP1, PARP, PARG, ATR and Chk1.
- BSM-1516 is a novel FEN1 inhibitor and is synergistic with inhibitors of USP1, PARP, PARG, ATR and Chk1.

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See recent poster from AACR poster (April 2024) at www.BlacksmithMedicines.com

Status and next steps

- BSM-1516 represents the most potent and selective FEN1 inhibitor
- Multiple synthetic lethal relationships and synergies with DDR drugs on market and in development
- *In vitro* ADME and *in vivo* PK studies demonstrate properties suitable for *in vivo* testing as a single agent or in combination; studies in process
- Recently unveiled program at AACR in San Diego

Summary

At Blacksmith Medicines, we are developing medicines targeting metal-dependent enzymes with a focus on Oncology and Infectious Disease

Near-term validation with LpxC IND and Phase 1

Blacksmith has executed strategic drug discovery collaborations with Lilly, Roche, Basilea, Cyteir, and Zoetis LLC., and has been awarded non-dilutive Federal funding agreements with CARB-X and NIH/NIAID

Continued support through non-dilutive contracts and milestones

Blacksmith investors include Lilly, Evotec, MP Healthcare, MagnaSci, and Alexandria Venture Investments

Novel oncology assets to drive significant value going forward

For further information, please visit the company's website www.BlacksmithMedicines.com and LinkedIn

Additional partnering opportunities as future growth catalysts

