Blacksmith Corporate Overview

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

BLACKSMITH Medicines

Needham Healthcare Conference
April 2024

Blacksmith Medicines: Executive summary

Mission:

To be the leading biopharma dedicated to discovering and developing medicines targeting *metallo*enzymes

- Purpose-built platform for *metallo*enzyme-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

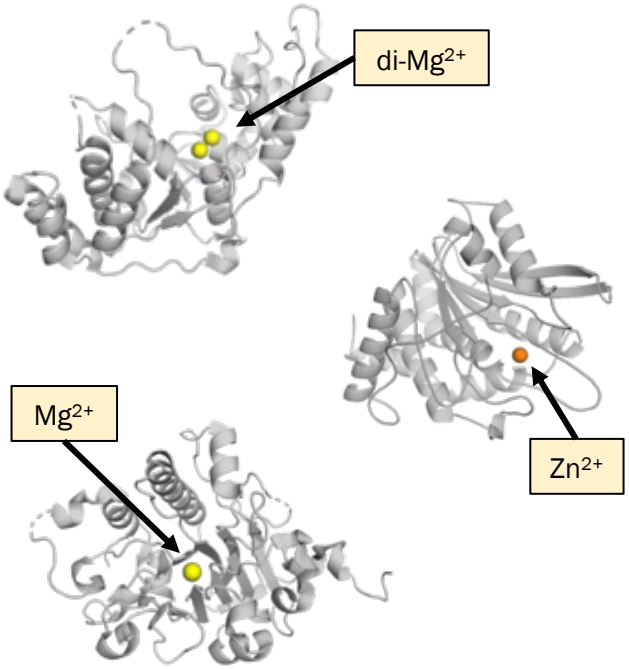
Introduction: Metalloenzymes & metalloenzyme inhibitors

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

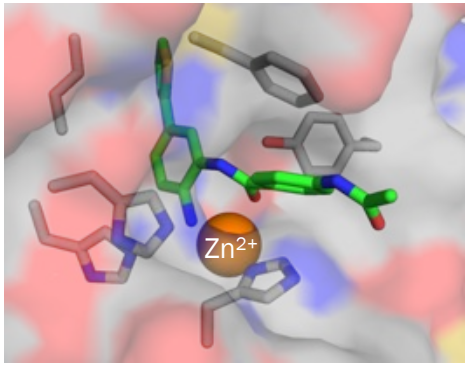
Metal ions like magnesium, zinc, iron, manganese, and copper are the essential ingredient in metalloenzymes



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

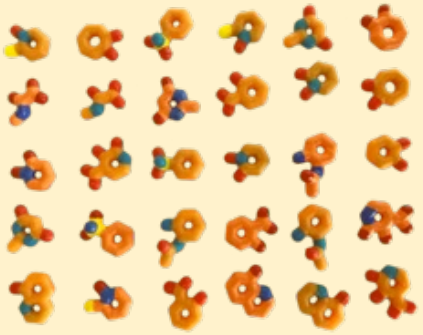
Pharma's libraries do not contain these types of metal binding fragments; we use the same pharmacophores that everybody else uses and when we change an atom to optimize or get around IP concerns we lose potency

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'

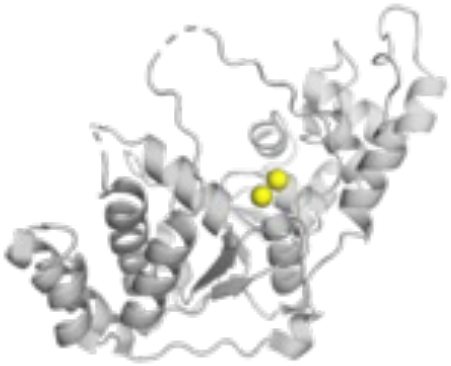


Key differentiation: Focused library of >1500 MBPs with various donor atom configurations and metal-binding characteristics (e.g. donor geometry, pK_a)

- *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

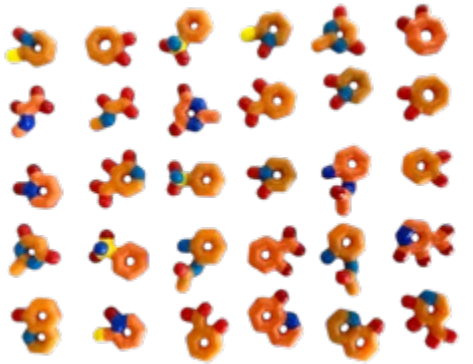
Solution: The power of fragments & key bioinorganic know-how to accelerate lead identification

Step 1: Establish biochemical assay



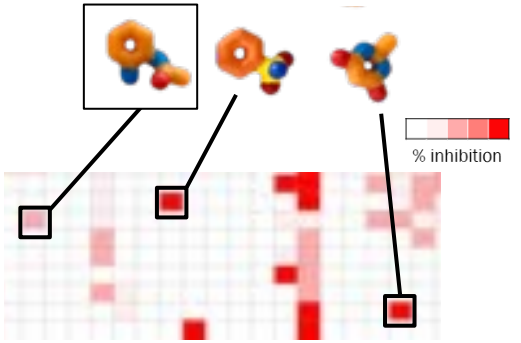
Metalloenzyme target

Step 2: Screen MBP library



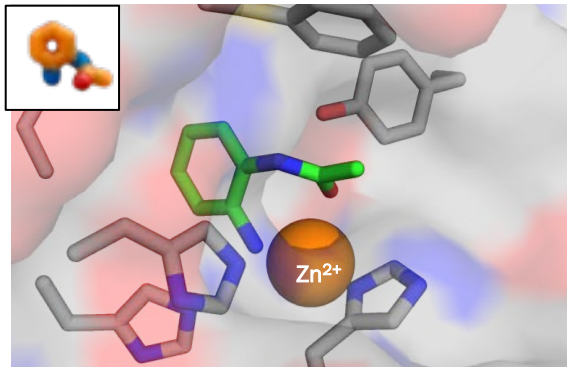
MBP fragment library

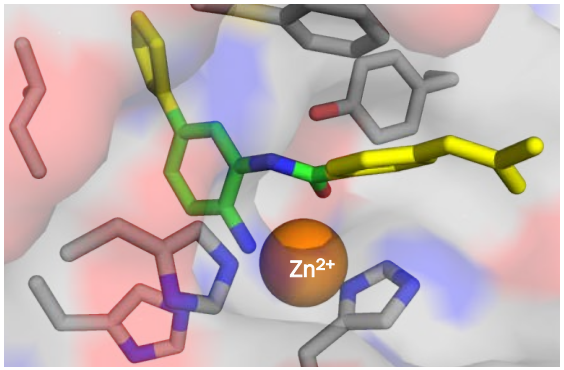
Step 3: Identify unique & selective MBP 'hits'



MBP inhibition heat map

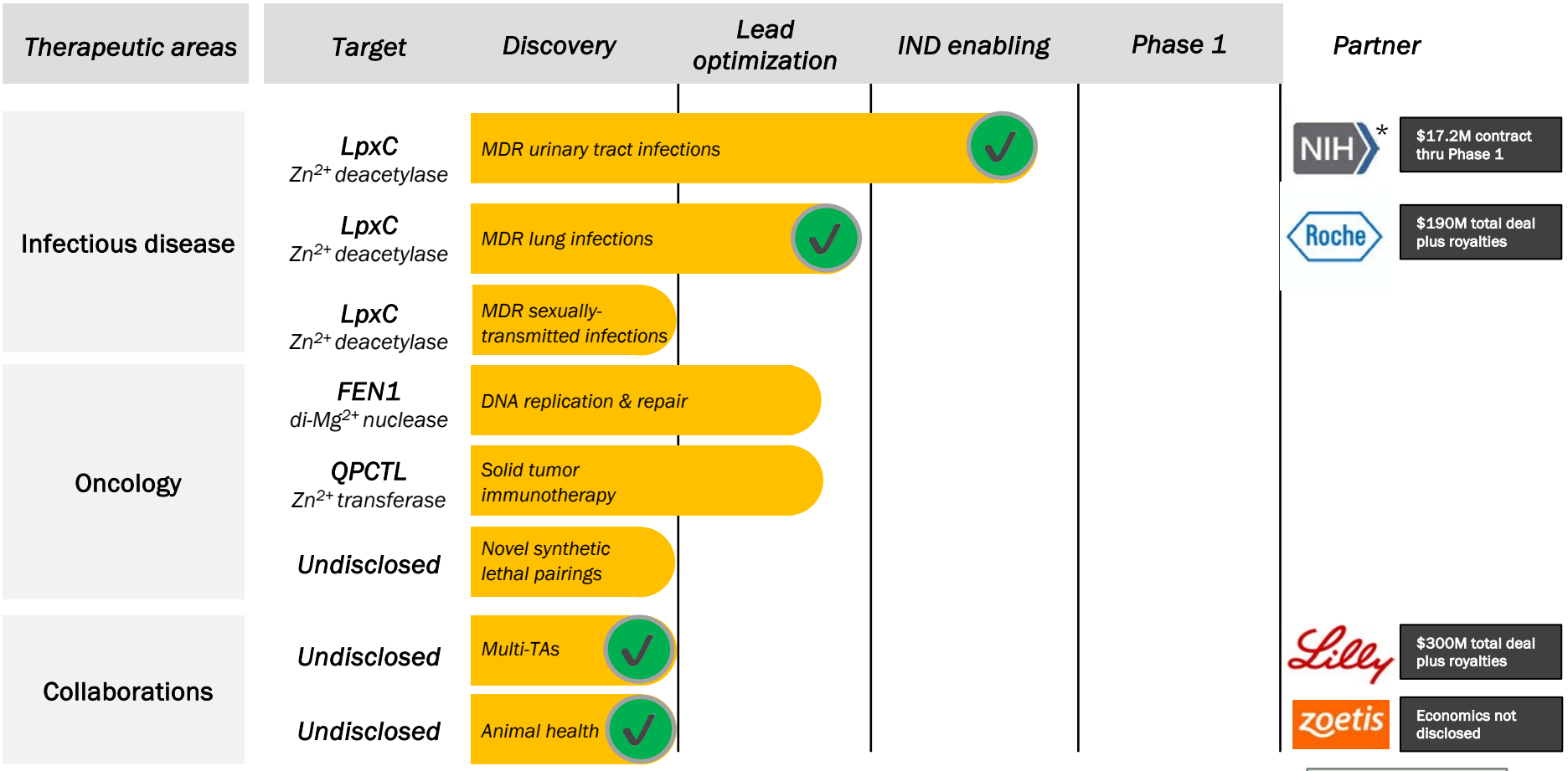
Step 4: Apply key bioinorganic know-how to model 'hits' with proper location & orientation followed by fragment growth strategy using Blacksmith's proprietary computational process





Lead ID

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



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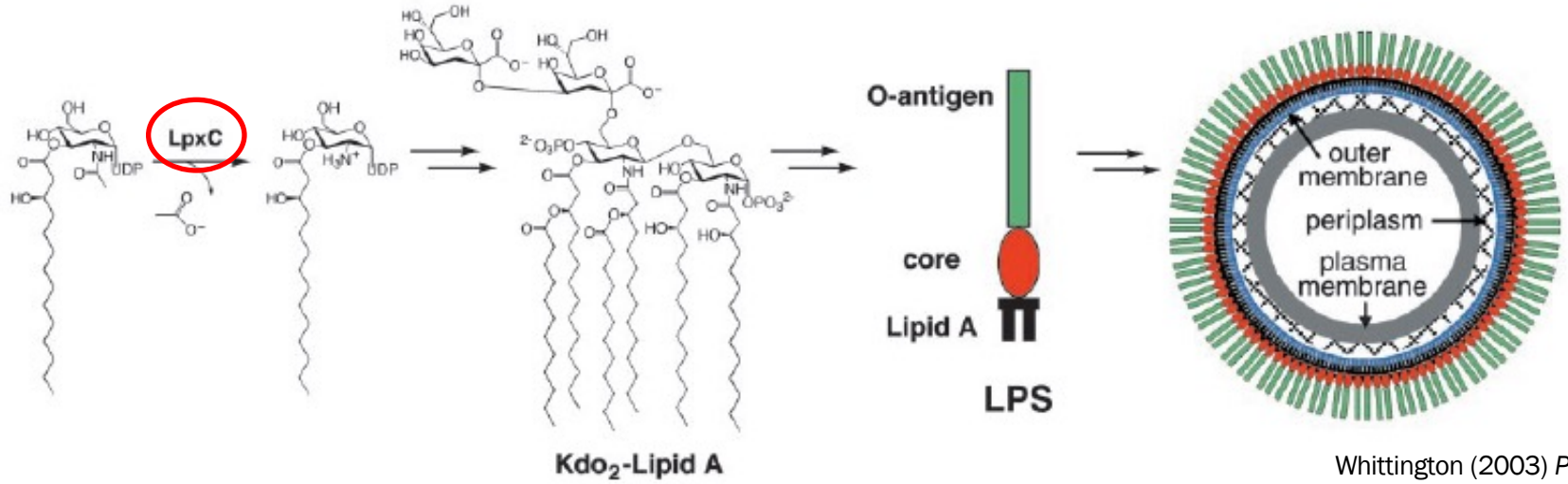
LpxC as a novel antibacterial target: Essential for biosynthesis of Gram-negative bacterial outer membrane



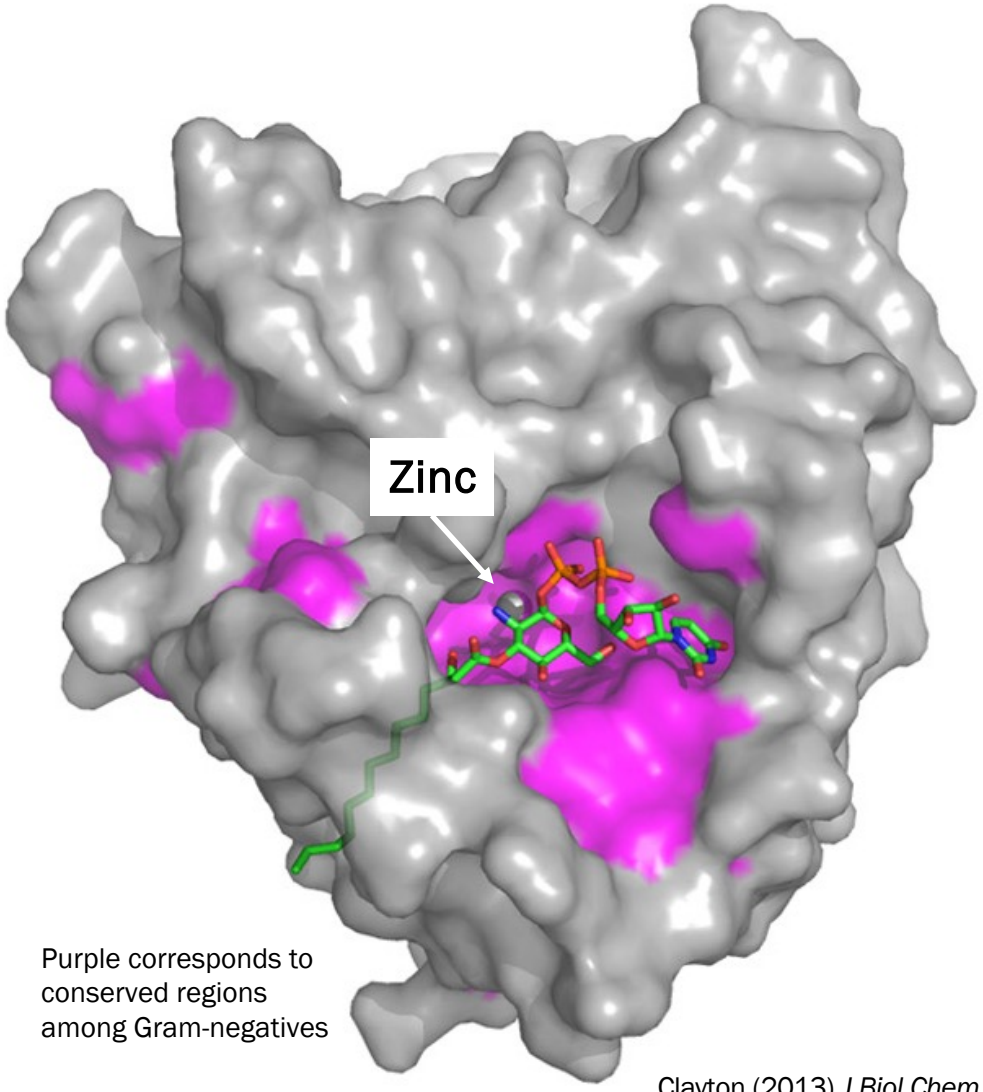
Whittington (2003) *PNAS*

- 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

Contents lists available at ScienceDirect

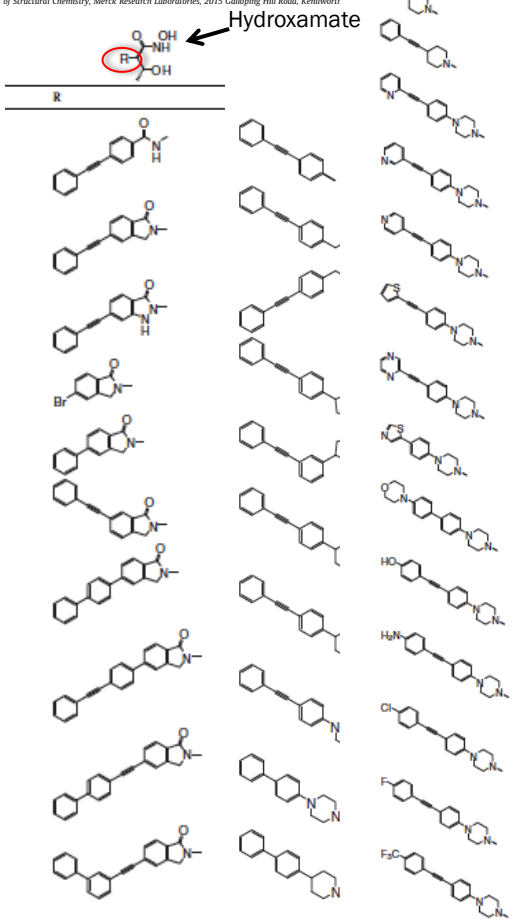
Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design and synthesis of potent Gram-negative specific LpxC inhibitors

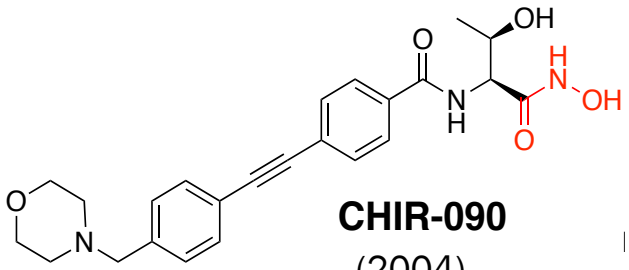
U. Faruk Mansoor[a,*], Dilrukshi Vitharana[a], Panduranga Adulla Reddy[a], Dayna L. Daubaras[b], Paul McNicholas[b], Peter Orth[c], Todd Black[b], M. Arshad Siddiqui[a]

[a] Department of Chemistry, Merck Research Laboratories, 320 Bent Street, Cambridge, MA 02141, USA
[b] Department of Biology, Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, NJ 07033, t
[c] Department of Structural Chemistry, Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth

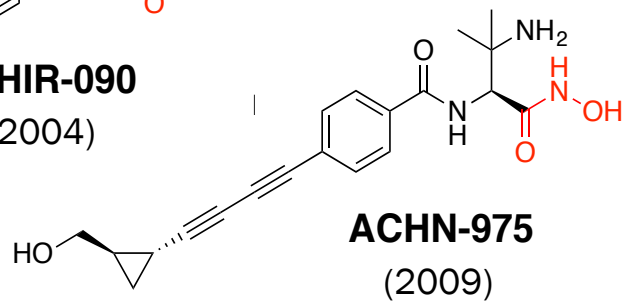


Merck 2011

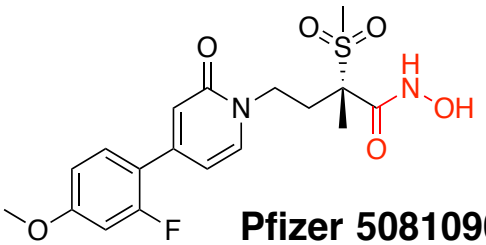
- Previous LpxC inhibitors relied on hydroxamate chemistry as MBP (in RED)
- Potent *in vitro*
- Suffer from metabolism, high clearance *in vivo* and cardiovascular toxicity
- No approved drugs



CHIR-090
(2004)



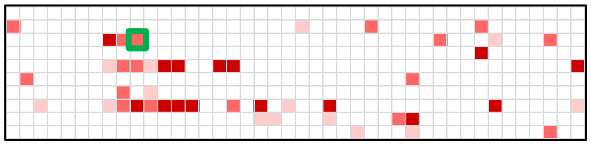
ACHN-975
(2009)



Pfizer 5081090
(2012)

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

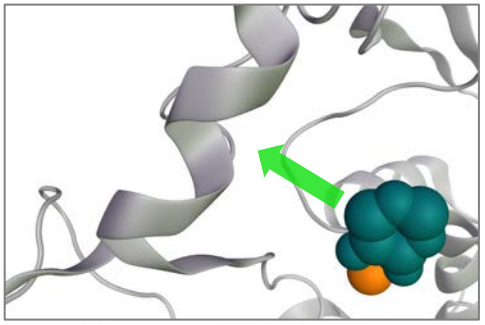
1 MBP screen identifies new starting points

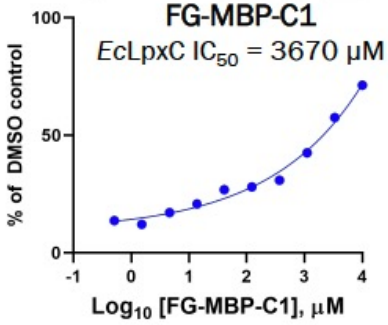


Program activities/achievements:

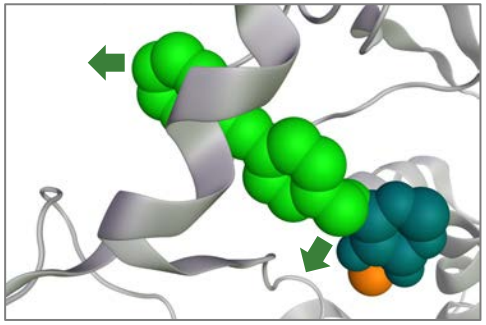
- ✓ Established enzyme assay
- ✓ Screened MBP library
- ✓ Verified MBP dose response
- ✓ Established structural biology
- ✓ Designed & synthesized full-length inhibitors
- ✓ Demonstrated *in vitro* activity w/MDR strains
- ✓ De-risked resistance liabilities
- ✓ Demonstrated *in vivo* efficacy and safety

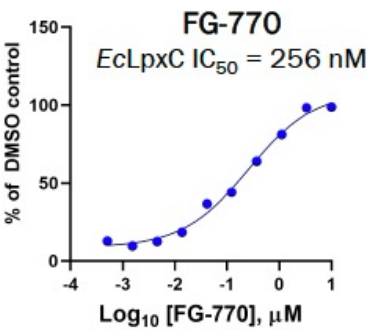
2 Bioinorganic modeling of MBPs to develop *metal binding hypothesis*



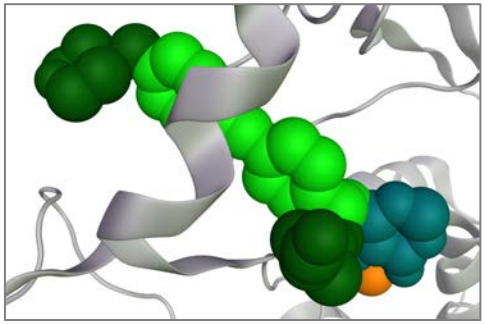


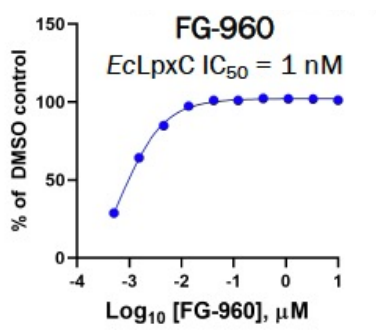
3 Elaborate MBPs/initiate computational to test





4 Chemistry campaign to **build desired potency/properties**



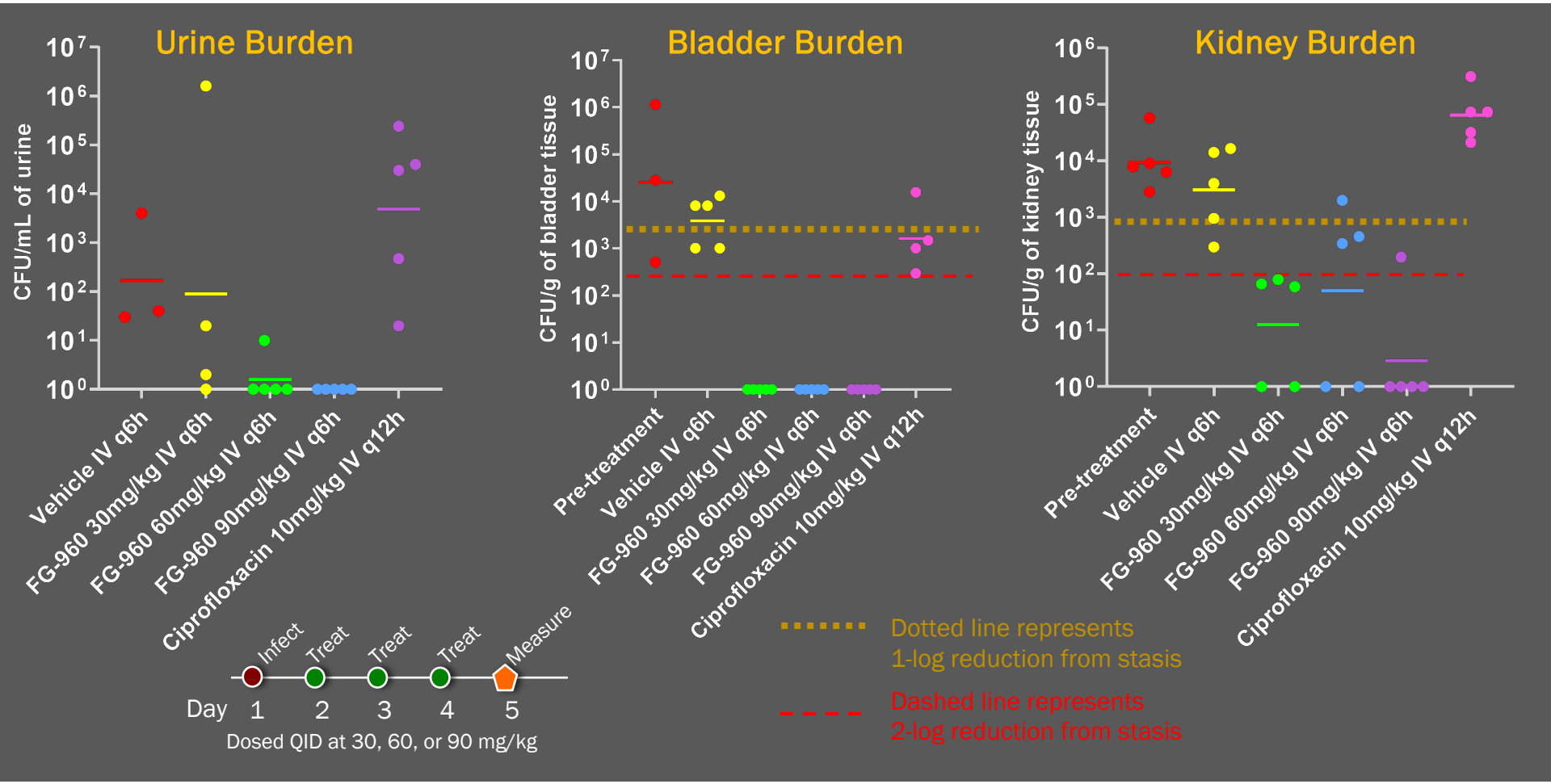


In vitro data: Potent Gram-negative bacteria killing

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

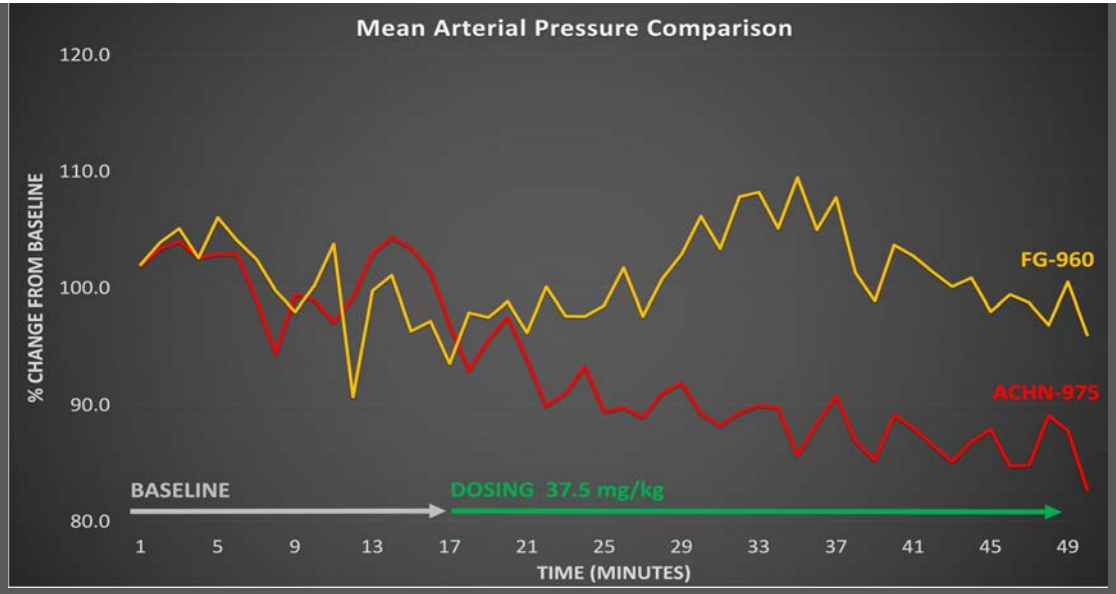
FG-960: Novel non-hydroxamate LpxC inhibitor able to kill drug-resistant Gram-negative bacteria

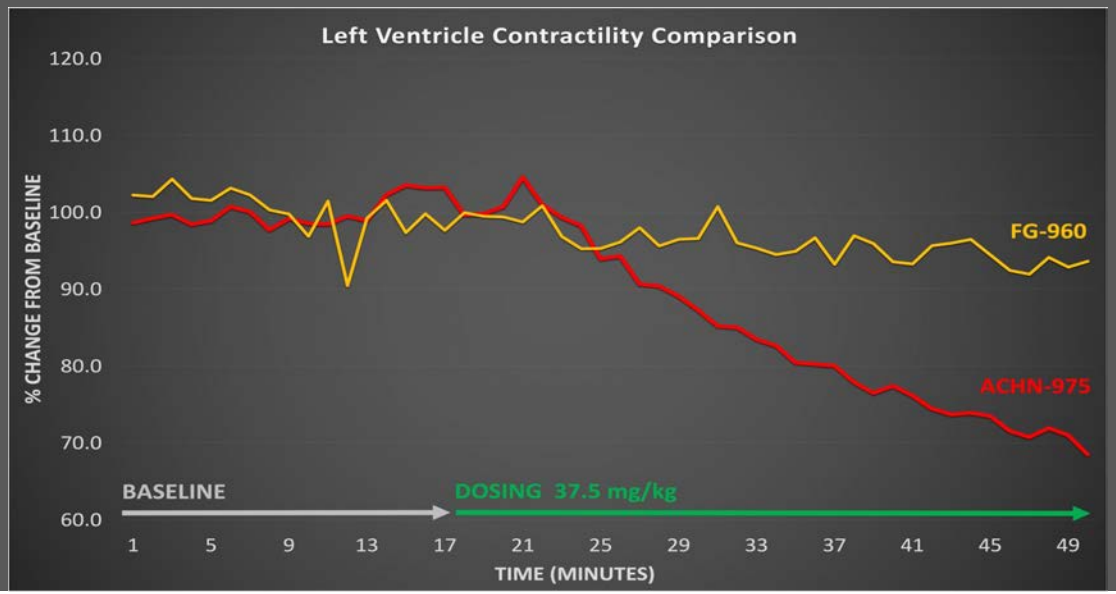
In vivo data: Efficacy against drug resistant *K. pneumoniae*



- 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

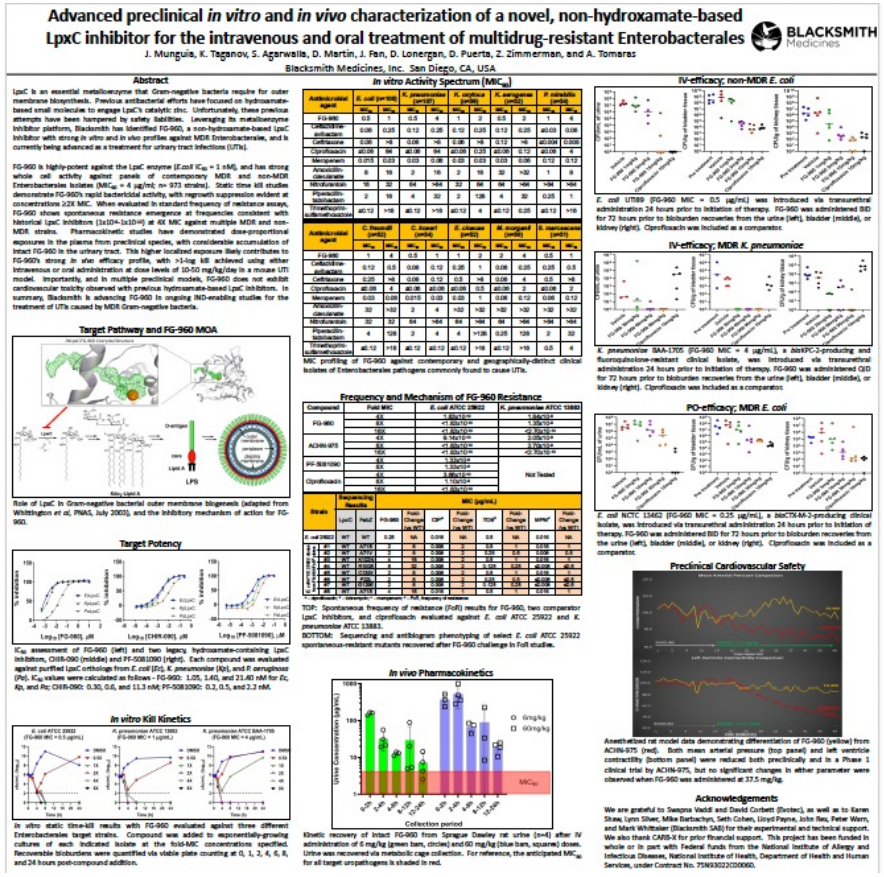




Differentiation: FG-960 does not induce hemodynamic effects in rats.

Both compounds dosed at 75 mg/kg IV

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



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

Status and next steps

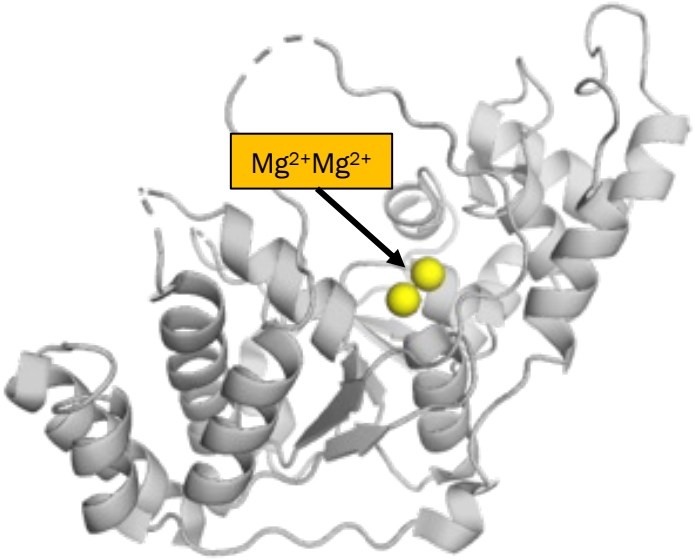
- Novel non-hydroxamate LpxC inhibitor single digit nanomolar potency
- *In vivo* efficacy in multiple infection models using IV and oral routes of administration
- Completed IND-enabling toxicology with no concerns
- Currently undergoing scale-up for clinical trials
- Fully-funded through the end of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

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

BLACKSMITH Medicines

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

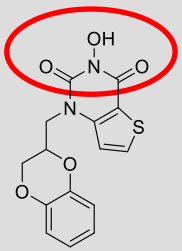
- FEN1 is highly conserved from yeast to humans indicating its important to cell biology
- FEN1 is highly expressed in proliferative tissue and even higher in cancer

Therapeutic relevance

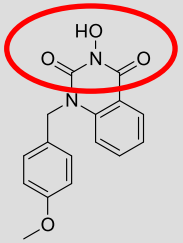
- Targeting FEN1 has been proposed to be an important therapeutic strategy alone or synergistic with current treatments, however, chemistry limitations have hampered progress

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

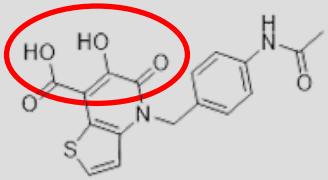
Mg^{2+} binding chemistry



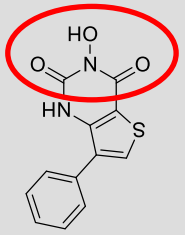
Cmpd 17



Cmpd 4



Cmpd 40



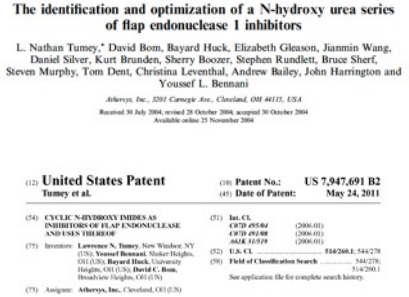
Cmpd C8

2005
Tumey *et al.*
Athersys

2016
Exell *et al.*
Astra Zeneca

2019
Ideaya
Biosciences

2020
Guo *et al.*
UC San Diego



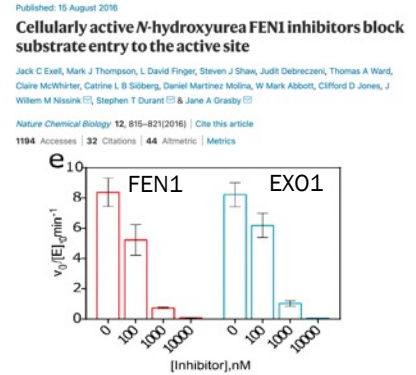
The identification and optimization of a N-hydroxy urea series of flap endonuclease 1 inhibitors

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

(12) United States Patent
Tumey et al.

(10) Patent No.: US 7,947,691 B2
(45) Date of Patent: May 24, 2011

(54) CYCLIC N-HYDROXY IMIDES AS INHIBITORS OF FLAP ENDONUCLEASE AND USES THEREOF



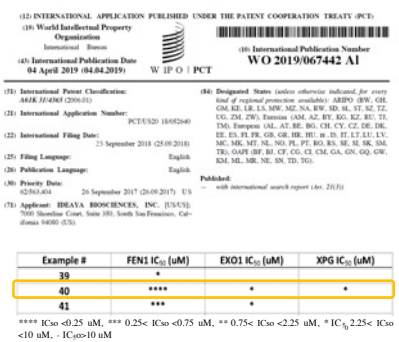
Published: 15 August 2016

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, Catrine L B Siöberg, Daniel Martinez Molina, W Mark Abbott, Clifford D Jones, J Willem M Nissink, Stephen T Durant & Jane A Grasby

Nature Chemical Biology **12**, 815–821(2016) | Cite this article

1194 Accesses | 32 Citations | 44 Altmetric | Metrics



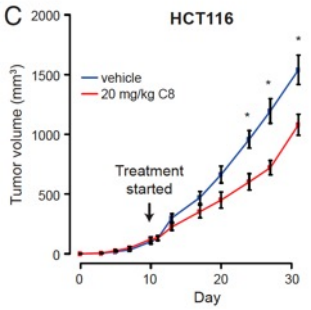
(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(10) International Publication Number
WO 2019/067442 A1

(43) International Publication Date
04 April 2019 (04.04.2019)

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, * IC_0 2.25< IC50 <10 uM, - IC50>10 uM



Guo E *et al. PNAS* 2020

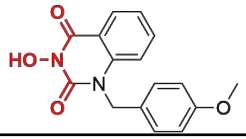
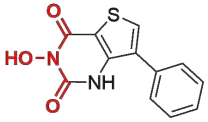
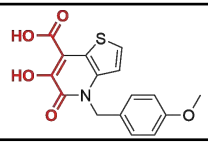
Athersys N-hydroxy urea patent issued in 2011

N-hydroxy urea inhibitors are not selective for FEN1

FEN1 selectivity observed but limited cell activity

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

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

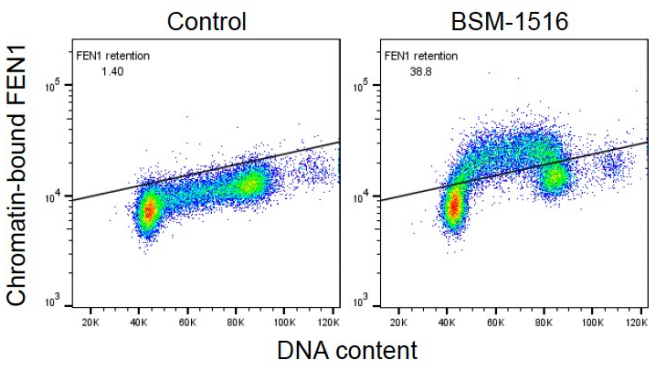
BSM-1516: To our knowledge, the most potent & selective FEN1 inhibitor to date
PLUS contains a novel MBPs and does not utilize an N-hydroxy urea or carboxylic acid

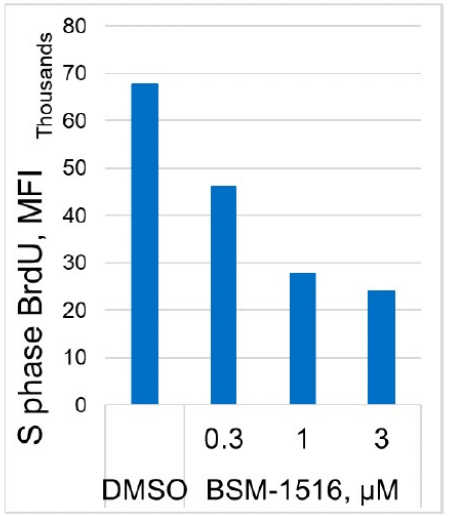
BSM-FEN1: Cellular target engagement and mechanism

FEN1 target engagement by cellular thermal shift assay

Company	ID	CETSA EC_{50} (μM)
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

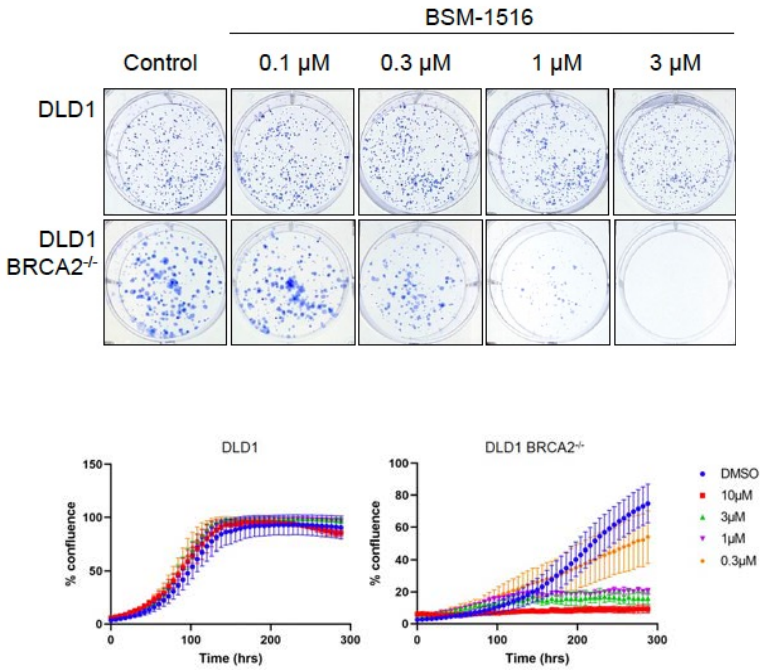




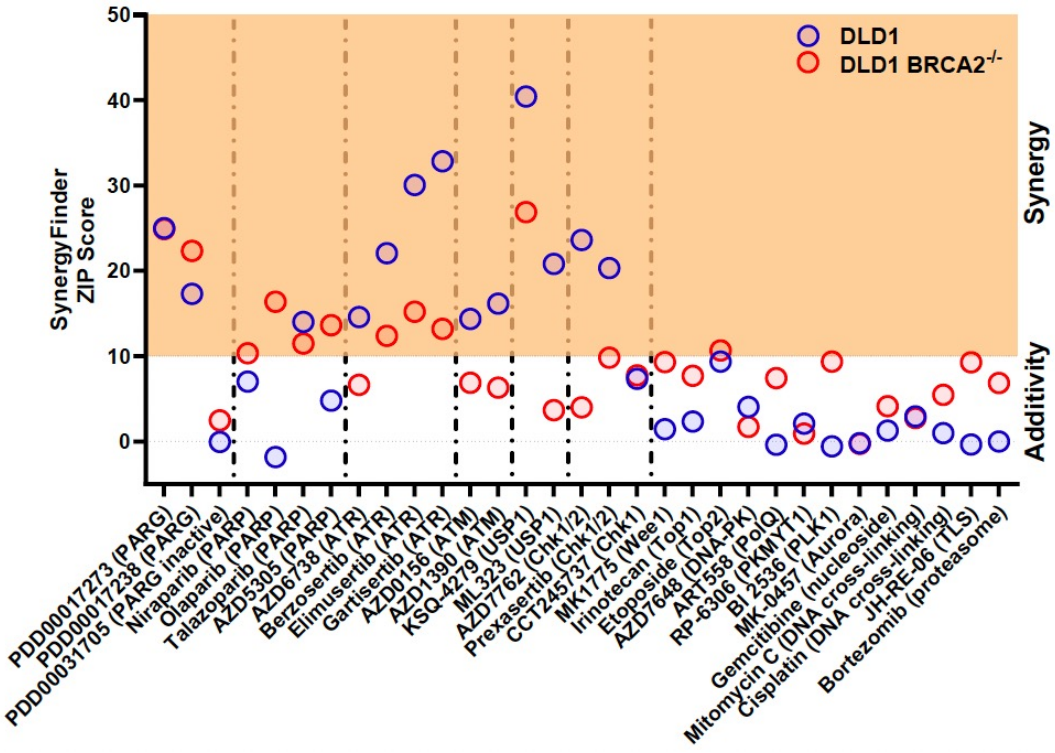
BSM-1516 stabilizes FEN1 and increases its chromatin retention in S phase that is accompanied by slowdown of DNA replication

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)

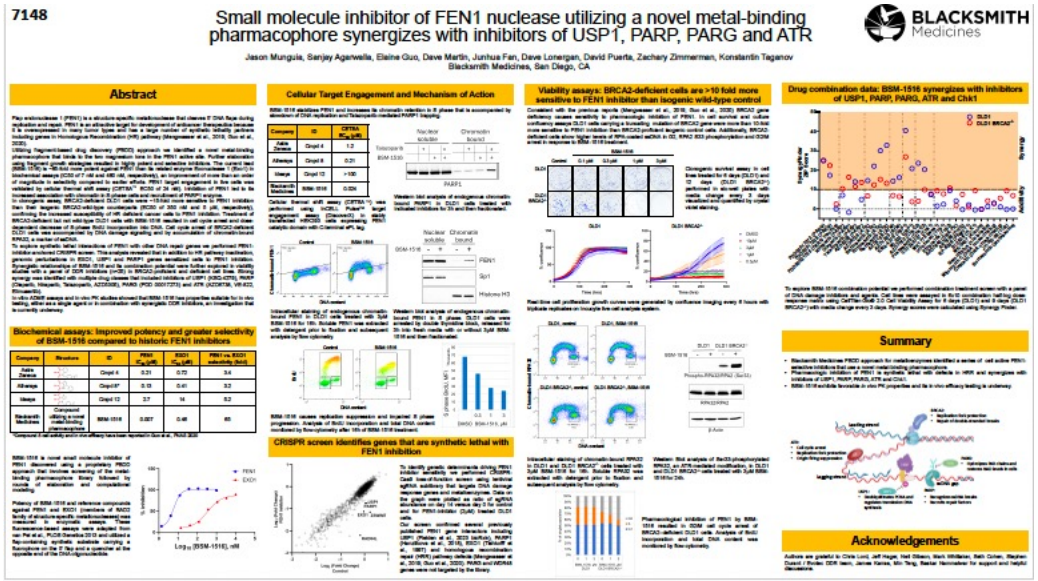


BSM-1516 shows strong synergy with multiple DDR drug classes on the market and in the clinic including inhibitors of PARP, PARG, USP1 and ATR



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

Jason Mungulia, Sanjay Agarwalla, Elaine Guo, Dave Martin, Junhua Fan, Dave Lonergan, David Puerta, Zachary Zimmerman, Konstantin Taganov
Blacksmith Medicines, San Diego, CA

BLACKSMITH Medicines

Abstract

Cellular Target Engagement and Mechanism of Action

Viability assays: BRCA2-deficient cells are >10 fold 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

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

CRISPR screen identifies genes that are synthetic lethal with FEN1 inhibition

Summary

Acknowledgements

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

BLACKSMITH Medicines