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Chemical Mimetics of Host Defense Proteins

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OTC: PYMX

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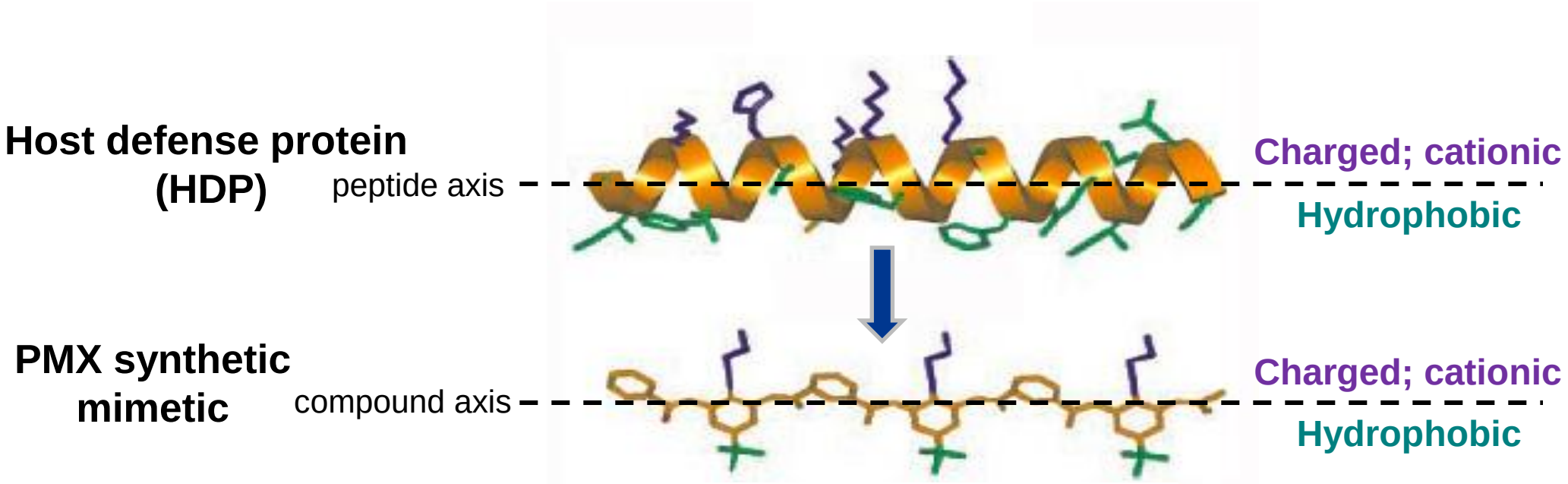
Antimicrobial Program; Background

- **Develop small non-peptidic, fully synthetic mimics of the Host Defense Proteins (HDPs) as systemic and topical antibiotics**
  - Novel approach for bactericidal activity
  - Clinical lead: Brilacidin (PMX-30063) in Phase 2 clinical study for ABSSSI
- **HDPs are small antimicrobial peptides**
  - Produced in skin, mucosal surfaces, neutrophils
  - Widespread in the animal kingdom
  - Target microbial membrane
- **First line of defense against bacterial invasion**
  - Part of innate immunity
  - Maintenance of epithelial barrier function
  - Regulate microbiota - disrupt bacterial cell membranes
  - Immuno-modulatory activities – link innate and adaptive immunity
- **Implicated in many diseases**
  - IBD, atopic dermatitis, acne, otitis media, cystic fibrosis…

Design Approach

The biological activities of host defense proteins depend on an *amphiphilic helix*



Capture structural and biological properties of HDPs using fully synthetic, nonpeptidic scaffolds and sidechains

Not peptidomimetics

Advantages: Mimetic Approach

- **Narrow and broad-spectrum antimicrobial agents have been produced**
  - 0.5 to 2 µg/ml MICs vs Gram-positives
  - 0.5 to 8 µg/ml MICs vs Gram-negatives
- **Wide selectivity for bacteria over mammalian cells**
  - Significant improvements in cytotoxicity versus HDPs
  - >100 to 1,000 fold selectivities
- **Medicinal chemistry enables "fine-tuning" for specific activities**
- **Straightforward synthesis**
  - Common starting materials
- **Metabolically stable and active *in vivo***
- **Developed for systemic and topical uses**

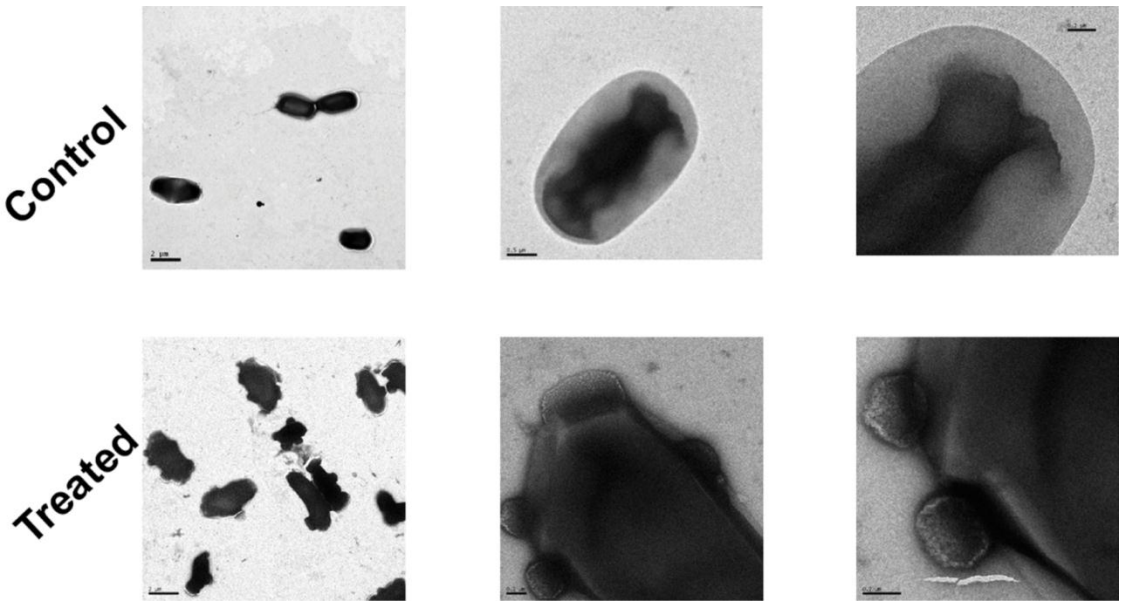
Features of the PMX Compound Library

- **Fully-synthetic, nonpeptidic mimics of HDPs**
  - Capture structure/function on small nonpeptidic scaffold
- **Multiple compound series; defined by the backbone**
  - arylamides; arylureas, tricyclics, phenylalkynes, salicylamides, triaryls
- **Restricted torsional freedom around axis critical**
  - Stabilize amphiphilic structure
  - Improves antimicrobial activity
- **Side chains (R1, R2, R3) provide high degrees of freedom for fine-tuning hydrophobicity and cationic balance**

Mechanism of Action: Membrane Target

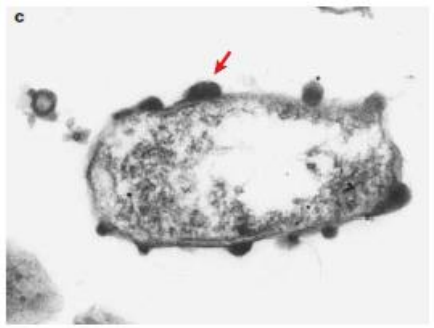
Membrane activity in Gram-positive and Gram-negative organisms supported by

- **Coarse grain molecular dynamic simulations**
- **Vesicle leakage assays**
- **Membrane permeabilization and potentiation assays**
- **Transcriptional profiling, proteomics and deep sequencing**
- **Transmission electron microscopy**



60 minutes; 10x MIC concentrations

TEM of *P. aeruginosa* on SMAP29 (3 hrs)



Brogden, K. 2005. Nature Reviews, Microbiology *3*: 238 (2005)

Cidal concs. of a HDP mimic cause visible signs of vesiculation (blebbing) at the E. coli membrane.

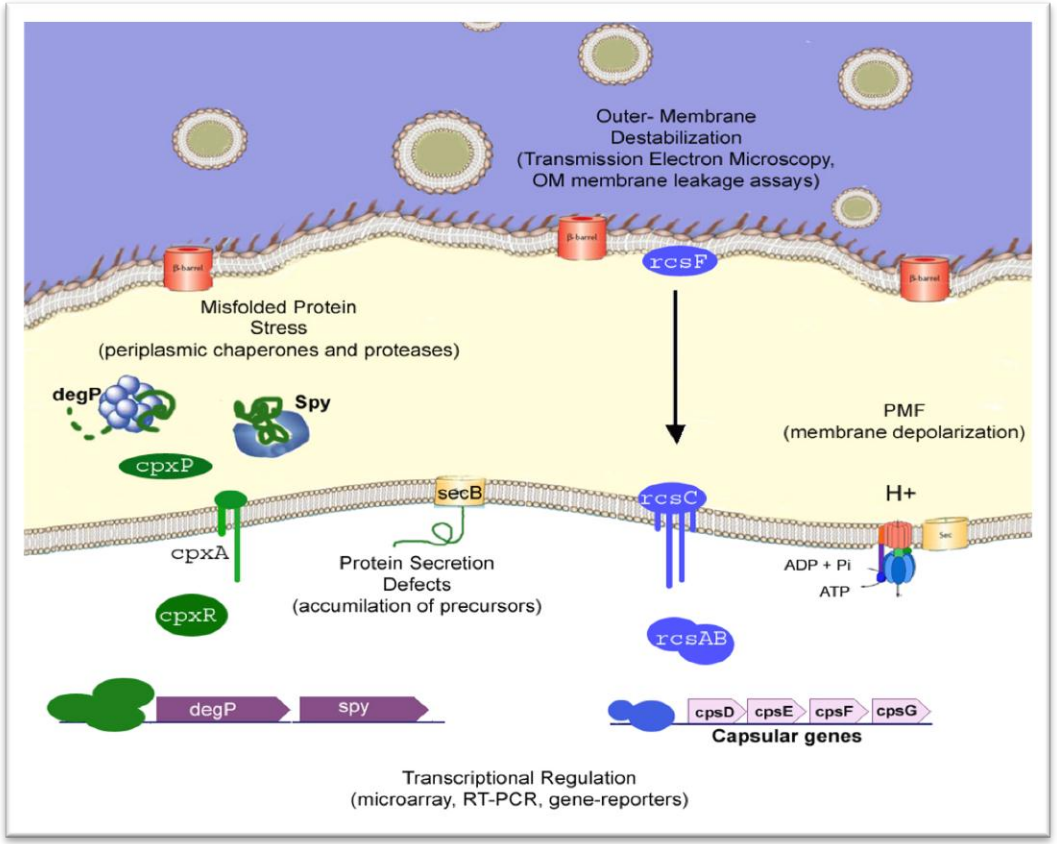
Similar morphological response reported for SMAP29 and P. aeruginosa.

Mensa et al. 2011 Antibacterial Mechanism of Action of Arylamide Foldamers; AAC In Press.

Current view on MOA for optimized arylamides

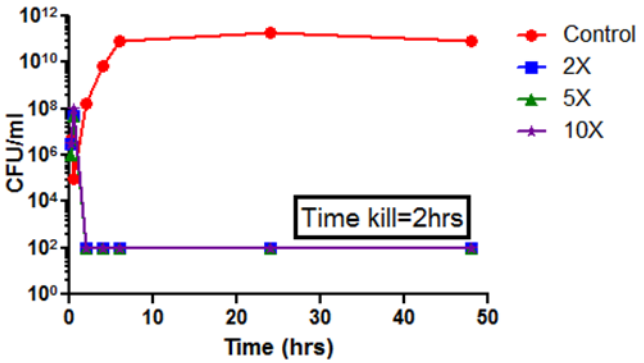
in collaboration with W. DeGrado (UCSF)

- **Muted membrane and capsule stress but no large-scale leakage from the cytoplasmic membrane**
  - **Damage/leakage evident in outer membrane of Gram-negative *E. coli***
- **Accumulation of unprocessed secreted proteins by mal-functioning translocon**
  - **Caused by change in membrane properties and/or plasma membrane depolarization**
- **Up-regulation of chaperones and proteases that target mis-folded proteins**
- **Blockade of protein secretion and/or accumulation of toxic aggregates leads to cell death**

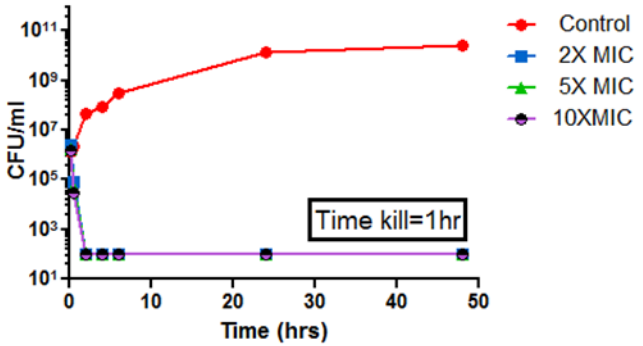


Antimicrobial Activities;
Rapid killing kinectics vs Gram+ and Gram- bacteria

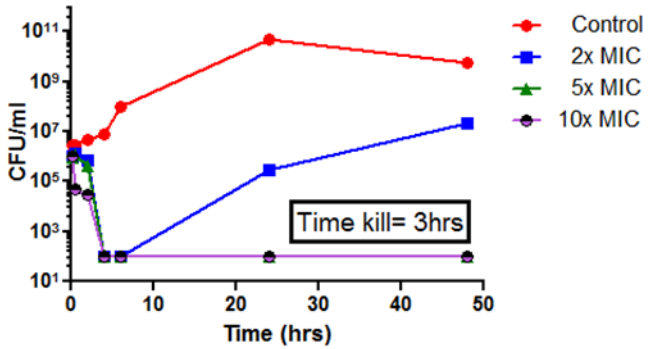
PMX183
E. faecalis ATCC 29212



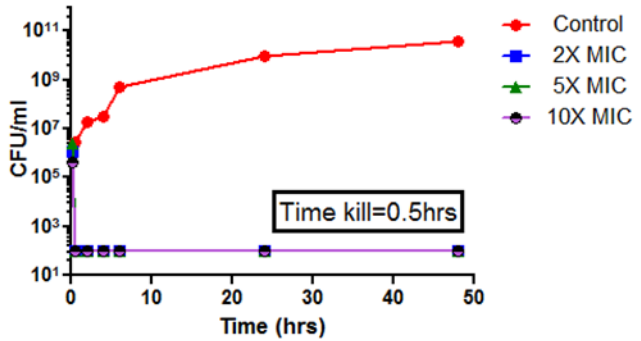
PMX519
S. aureus BAA-1556



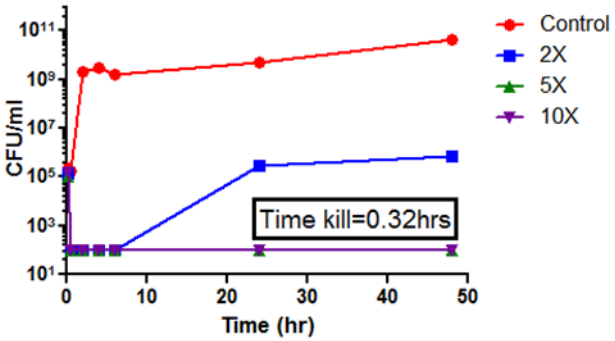
PMX668
S. aureus BAA-1556



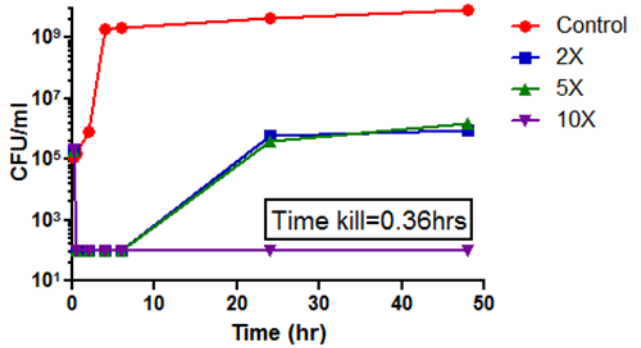
PMX100
S. epidermis ATCC 12228



PMX519
A. baumanii ATCC 19003



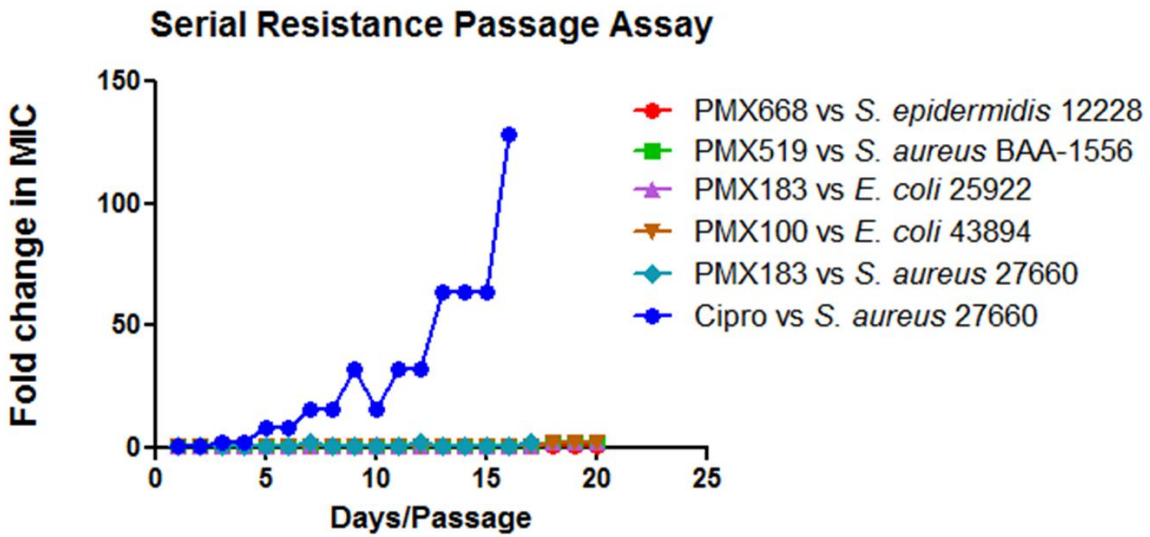
PMX668
P. aeruginosa ATCC 10752



Antimicrobial Activities;
Low resistance potential

Spontaneous resistance frequencies are < 10^{-11} with Gram-positive and Gram-negative bacteria

Antibacterial	MIC	Mutation Frequencies		
		S. aureus 29213	*E. coli* 25922	*S. enterica* 4931
Brilacidin (PMX30063)	1x	< 1×10^{-11}	< 1×10^{-11}	< 1×10^{-11}
PMX183	3x	< 1×10^{-10}	< 1×10^{-10}	< 1×10^{-10}
Ciprofloxacin	3x	5×10^{-9}	1.4×10^{-9}	NT
Gentamycin	3x	2.25×10^{-7}	5×10^{-8}	5×10^{-8}
Daptomycin	3x	1×10^{-10}	NT	NT
NT: Not Tested				



No emergence of resistance over 20 passages with Gram-positive and Gram-negative bacteria

Susceptibility of *Staphylococcus* isolates with varying drug-resistance phenotypes

Relative to oxa-S isolates, susceptibility not affected by other drug-resistance phenotypes

Drug Phenotype	# isolates	MIC Range (µg/ml)				
		PMX100	PMX183	PMX519	PMX668	PMX141
S. aureus OXA-S						
OXA-S	50	0.5 - 1	≤0.25 - 1	≤0.25 - 0.5	≤0.25 - 1	0.25 - 2
DAP-NS	1	0.5	0.5	0.5	0.5	0.5
S. aureus OXA-R (MRSA)						
OXA-R	50	0.5 - 1	0.5 - 1	≤0.25 - 0.5	≤0.25 - 0.5	0.5 - 1
VRSA	3	0.5	0.5	0.5	≤0.25	≤0.25 - 0.5
VISA	3	0.5	0.5	0.5	≤0.25 - 0.5	≤0.25 - 0.5
LIN-NS	3	0.5 - 1	0.5 - 1	0.5	0.5	≤0.25 - 0.5
DAP-NS	2	1	0.5	0.5-1	0.5	0.5 - 1

Presentation Topics

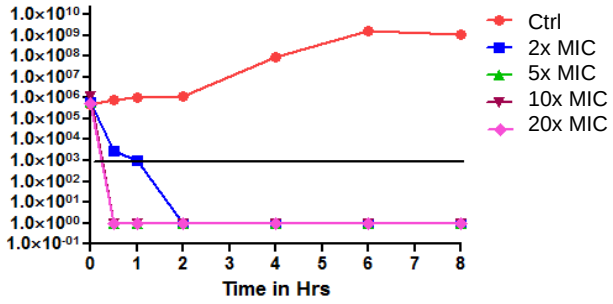
- **Brilacidin (PMX-30063); Clinical status**
- **Preclinical development of antimicrobial therapeutics for:**
  - **Gram-negative pathogens**
  - **Fungal pathogens**
  - **Malaria**
- **Oral mucositis; Brilacidin**

Brilacidin: Activity Profile
in vitro

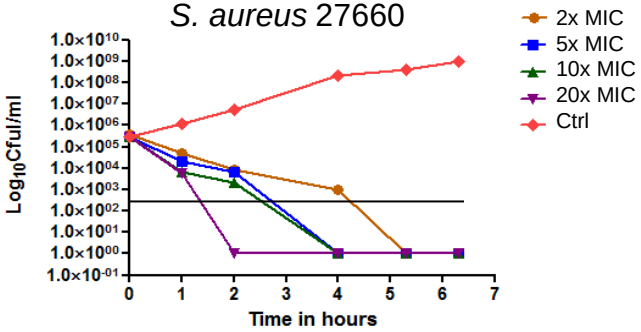
Cmpd	MIC90s (µg/ml)			MIC range (µg/ml) 2 – 3 clinical isolates			Cytotoxicity (EC_{50}, uM)		
	MSSA	MRSA	CoNS	*E. coli*	*K. pneumoniae*	*Enterobacter* spp.	RBCs	3T3	HepG2
Brilacidin	1	1	0.5	1 - 2	1 - 4	0.5 - 4	>500	430	1,031

Gram+ activity,
Gram- coverage,
Low cytotoxicity

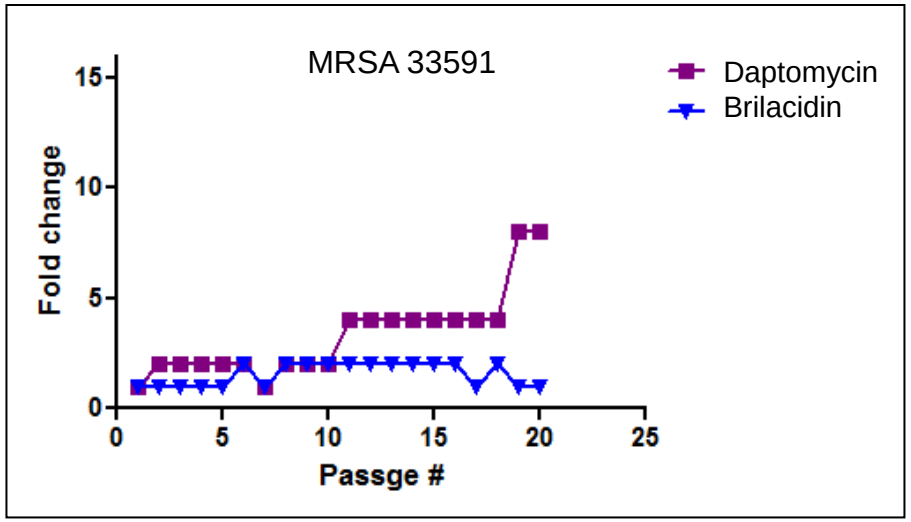
E. coli D32



S. aureus 27660



Rapid bactericidal activity;
0.5 to 4-6 hrs.



Low risk for resistance development
FSR < 10^{-11} @ 3x MIC

Brilacidin vs *Staph*. spp. with Defined Drug Susceptibility Phenotypes

Broad spectrum activity against multi-drug resistant strains of S. aureus, S. epidermidis and S. hemolyticus

	Drug-suscept.	OXA-R	VRSA/VISA OXA-R	LZD-NS OXA-R	DAP-NS OXA-R	VRSA/VISA DAP-NS OXA-R
isolates	**217**	**161**	**7**	**5**	**5**	**3**
Brilacidin MIC range	**0.25 - 1**	**0.25 - 2**	**0.5 - 1**	**0.5 - 1**	**0.5 - 2**	**0.5 - 1**

OXA-R: oxacillin-resistant
VISA: vancomycin intermediate S. aureus
VRSA: vancomycin resistant S. aureus
LZD-NS: linezolid non-susceptible
DAP-NS: daptomycin non-susceptible

Brilacidin: Pharmacological Properties

- **Gram-positive activity; some Gram-negative coverage**
- **Rapid bactericidal activity; 0.5 – 6 hr. time-kills**
- **Low resistance potential**
- **Suitable and predictable PK properties**
- **Long post-antibiotic effect *in vivo;* 14 – 16 hours**
- **Metabolically stable**

PK/PD parameters derived from animals models: C_{max} and AUC drivers for efficacy

Pathogen	Amt. of drug needed to achieve maximum-efficacy in animal-models *(AUC_{free} hr*ug/ml)*	Human equivalent drug exposure *(AUC_{free} hr*ug/ml)*	Human single dose *(mg/kg)*	Phase 1 Total Maximum Dose (mg/kg)
MSSA[1]	0.5 – 1.3	1.6	0.15	**2.5** (1 day)
MSSA[2]	1.9	2.2	0.20	**3.0** (over 5-10 days)
MRSA[1]	1.7 – 3.0	2.8	0.25	**5.55** (over 14 days)

[1] mouse thigh burden; [2] rat granuloma pouch

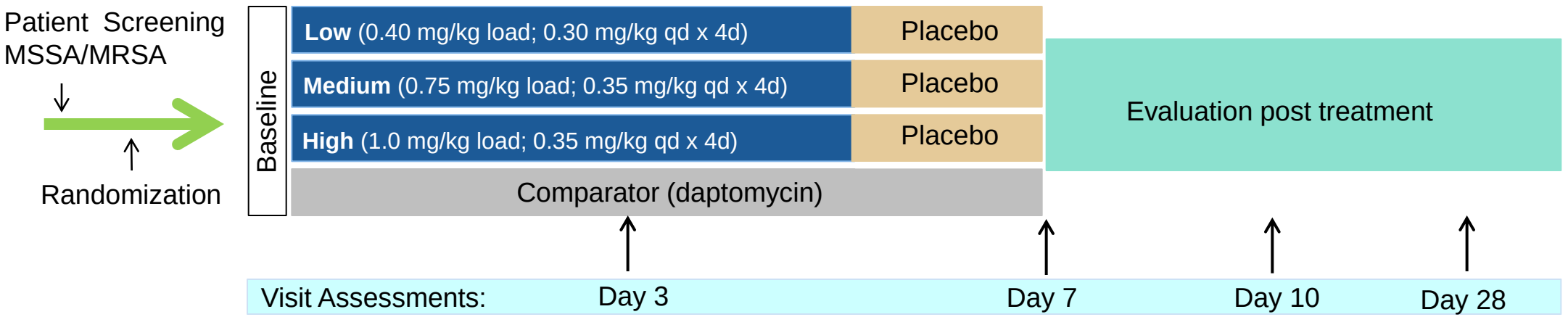
Brilacidin: Clinical Development for ABSSSI caused by *S. aureus*

- **Three phase 1 single and multi-dose studies completed**
  - Ascending single (PMX63-101) and multiple doses for 5 days (PMX63-102) and fixed dose for 14 days (PMX63-103)
  - IV infusions 48H, 24H and 12H
- **Pharmacokinetics**
  - Consistent and linear pharmacokinetics in plasma with half-live of ~ 23 hours. No apparent gender difference in drug disposition. Excretion primarily through fecal route.
- **Safety**
  - Paraesthesia and hypoaesthesia (numbness and tingling) with rapid resolution at end of treatment; No neurotoxicity evident in human subjects or animal safety studies.
  - Increase in blood pressure and heart rate reversible after treatment discontinuation
- **Phase 2 in ABSSSI**
  - One Phase 2A study completed
  - www.clinicaltrials.gov: NCT01211470

Phase 2 Clinical Trial – Design

Follows latest FDA guidance

L1-1662: Tuesday 11 AM



Continuous safety assessments and analysis

- **Trial conducted in Canada & Europe (Russia, Ukraine)**
- **215 patients, 4 arms, ~50 patients per arm**
- **Dosing: IV infusion 1x/day for 7 days (5 days on brilacidin + 2 days placebo; 7 days on daptomycin)**
- **Clinical assessments made on Days 3, 7, 10 and 28**

Clinical Response Rates

Per Protocol (PP) Population (n=161)

Study Visit	Low dose n=40	Medium dose n=35	High dose n=39	Daptomycin n=47
Day 2-3*	85.0%	71.4%	89.7%	74.5%
Day 2-3**	97.5%	91.4%	92.3%	91.5%
Day 7-8**	92.5%	94.3%	97.4%	95.7%
Day 10-14**	92.5%	91.4%	97.4%	95.7%
Day 28**	87.5%	80.0%	97.4%	93.6%

* FDA-defined clinical response; ** Sponsor-defined clinical response; Clinical response = significant reduction in lesion size at all assessment visits (no fever criteria)

- Clinical response rates were high across all 3 dose groups and at all time points and similar:
  - to the active control, daptomycin
  - across all analysis populations
    - PP, ITT, mITT
  - at Day 3 using the FDA or sponsor definitions
- Phase 2B study for dose optimization

Presentation Topics

- **Brilacidin; Clinical status**
- **Preclinical development of antimicrobial therapeutics for:**
  - **Gram-negative pathogens**
  - **Fungal pathogens**
  - **Malaria**
- **Oral mucositis; Brilacidin**

Gram-Negative Activity

- **6 Distinct series active against target pathogens with low cytotoxicity**
  - *E. coli, K. pneumoniae, P. aeruginosa and A. baumannii;* MICs 3.13 µg/ml or less
- **Significant advances in defining structure-activity relationships**
  - Balance of lipophilicity (LogD) and the number of positive charges
  - Incorporation of heteroatoms in side chain linkages and limitation of the torsional degrees of freedom

PMX	Series	MIC (µg/mL)							Cytotoxicity EC_{50} (µM)		MTD (mg/kg)
		A. baumannii		*P. aeruginosa*		*K. pneumoniae*		*E. coli*			
		BAA747	19606	10145	27853	13883	700603	25922	3T3	HG2	
100	Urea	3.13	12.5	3.13	0.78	0.78	6.25	0.78	128	145	17
229	Aryl Amide I	3.13	25	6.25	3.13	0.78	3.13	1.56	727	684	20-26
519	Aryl Amide II	1.56	6.25	1.56	0.78	0.78	0.78	0.78	430	>1000	17
633	Tricyclic	3.13	3.13	3.13	1.56	0.78	3.13	3.13	131	100	6.4
1091	Aryl Amide II	1.56	6.25	3.13	1.56	1.56	3.13	3.13	389	724	19-26
1142	Urea	3.13	>25	0.78	0.39	1.56	3.13	3.13	246	225	15
1241	Triaryl	6.25	12.5	3.13	3.13	0.39	1.56	3.13	115	138	5.7
1442	Aryl Amide II	3.13	6.25	6.25	3.13	0.78	0.78	0.78	181	601	40
1445	Aryl Amide II	3.13	12.5	3.13	1.56	6.25	12.5	3.13	973	>1000	20-30
1555	Benzimidazole	3.13	>25	3.13	1.56	3.13	3.13	3.13	102	391	20

Susceptibility of Drug-R Pathogens; ndm-1 *K. pneumoniae*

Compound	MICs (µg/ml) vs. *K. pneumonia* strains		
	NDM-1 (BAA 2146)	2 ATCC strains (non-NDM-1)	5 clinical isolates (non-NDM-1)
PMX868	0.78	0.39	ND
PMX1090	0.78	0.78	ND
PMX100	1.56	0.39	1 - 4
PMX223	1.56	0.78	1 - 2
PMX225	1.56	1.56	2 - 4
PMX183	3.13	0.39	1 - 2
PMX668	3.13	1.56	4
PMX519	3.13	1.56	2
PMX30063	3.13	0.78	1 - 2
polymyxin B	0.78	ND	ND
tigecycline	6.25	ND	ND
ceftriazone	>100	ND	ND
meropenem	>100	ND	ND

ND: Not Done

NDM-1 phenotype does not influence susceptibility to HDP mimics

Susceptibility of Drug-R Pathogens; *E.Coli* O104:H4; 2011 Germany Outbreak

PMX Compound	Series	O104:H4 Clinical Isolates (2)* MIC (µg/ml)		Cytotoxicity EC_{50} (µM)	
		BAA – 2326	BAA – 2309	3T3	HG2
PMX100	AR	0.78 – 1.56	1.56 – 3.13	128	145
PMX183	AA	0.78 – 3.13	1.56 – 3.13	139	227
PMX223	AA	0.78 – 3.13	0.78 – 3.13	178	480
PMX30063	AA	0.78 – 1.56	0.78 – 3.13	727	684
PMX247	AA	1.56	0.78 – 3.13	27	71
PMX519	AA	1.56	0.78 – 3.13	430	1000
PMX843	AA	1.56	1.56 – 3.13	79	131
PMX1091	AA	1.56 – 3.13	1.56 – 3.13	389	724
PMX1099	AR	1.56	1.56	57	106
PMX1278	TA	0.39 – 0.78	0.78	192	>1000
PMX1363	AA	0.78 – 1.56	1.56 – 3.13	422	262
PMX1405	BZ	1.56	0.78 – 1.56	>1000	913

AA: Arylamide; AR: Arylurea; TA: Triaryl; BZ: Benzimidazole; MICs (µg/mL); 3T3: mouse fibroblast (EC_{50} µM); HG2: human transformed liver cell (EC_{50} µM)
* USDA

Potent activity against enteroaggregative, shiga toxin-producing *E. coli* evident across multiple structural series with low cytotoxicity

HDP mimics; Category A and B Biopathogens

Compound	MIC (µg/ml); 30 isolates/organism									
	B. anthracis		*F. tularensis*		*Y. pestis*		*B. mallei*		*B. pseudomallei*	
	MIC_range	MIC_{90}	MIC_range	MIC_{90}	MIC_range	MIC_{90}	MIC_range	MIC_{90}	MIC_range	MIC_{90}
Existing Compound Library										
PMX0196	1 - 2	2	0.25 - 2	2	4 - 16	16	2 - 32	16	NT	NT
PMX0231	0.5 - 2	2	0.5 - 8	2	4 - 16	16	2 - 32	16	>32	>32
PMX0243	0.5 - 2	1	0.25 - 16	2	2 - 16	16	1 - 32	4	>32	>32
PMX0225	1 - 2	2	0.5 - 8	2	4 - 16	16	2 - 32	16	>32	>32
Optimized Compounds										
PMX1014	0.25 – 4	1	25-32	16	0.5 – 32	32	0.5 – 16	8	0.5 – 8	2
PMX1045	0.25 – 16	1	0.25 – 2	0.5	1 – 32	2	0.5 – 16	2	0.5 – 32	24
PMX1056	0.5 – 8	1	0.5 – 8	1	1 - 8	2	1 – 16	2	0.5 – 32	32
PMX1057	0.25 – 8	1	0.5 – 8	1	1 – 8	4	0.5 – 16	2	1 – 32	32
PMX1090	0.25 – 16	1	0.25 – 2	0.5	0.25 – 8	1	0.5 – 8	1	0.5 – 32	32
PMX1091	0.12 – 4	1	0.12 – 4	0.5	0.5 – 4	1	0.5 – 8	2	0.5 – 32	32
PMX1094	0.5 – 4	1	0.25 – 2	1	1 – 16	4	0.5 – 16	4	0.5 – 32	4
PMX1099	0.25 – 2	0.5	0.1 – 4	1	0.25 – 2	1	0.25 – 8	2	0.25 – 32	32
PMX1102	0.12 - 2	0.5	0.12 - 4	0.5	0.25 - 16	4	0.25 - 16	1	0.25 – 32	2

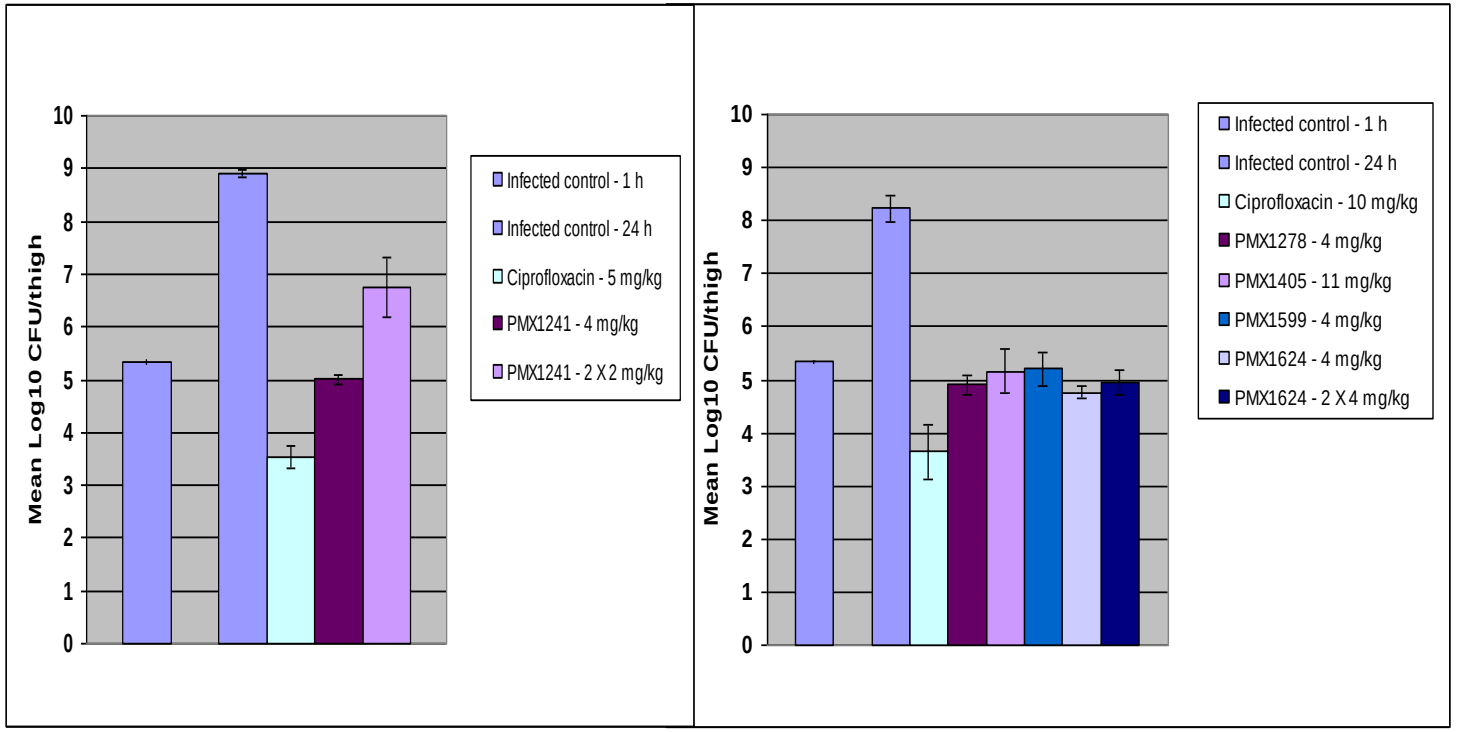
Screens performed by laboratories of H. Heine and C. Marchand; USAMRIID, Ft. Detrick

Library screen: Activity vs. *B. anthracis*, limited coverage over Gram-negative pathogens

Chemical optimization: Improved Gram-negative coverage

Animal Efficacy vs. *E. coli*; Mouse Thigh Burden model

- **Focus placed on potent activity in serum to enhance prospects for *in vivo* efficacy**
  - Screening model for in vivo efficacy: Mouse thigh burden
  - Promising new series showing potent serum activity identified; the triaryls
  - PMX1241 and 3 other triaryls are highly active vs. *E. coli* in the thigh burden model
  - Threshold for *in vivo* activity against *E. coli* appears to be MICs < 6 µg/ml in serum



Model:

- T = 0; neutropenic CD-1 mice are infected with 1.3 x 10^5 cfus E. coli 25922 in thigh muscles
- T = 1 hr: test agent administered IV 1x or 2x/day
- T = 24 hrs: Thighs are harvested for quantitation of tissue burden

Presentation Topics

- **Brilacidin; Clinical status**
- **Preclinical development of antimicrobial therapeutics for:**
  - **Gram-negative pathogens**
  - **Fungal pathogens**
    - **Oral candidiasis**
    - **Disseminated fungal infections**
  - **Malaria**
- **Oral mucositis; Brilacidin**

Lead compounds; Additional Properties

Library screen and chemical optimizations have identified compounds

- highly potent vs. *C. albicans* and non-albicans Candida (NAC) species
- with low cytotoxicity

Several leads show low anti-bacterial activity

- Commensal bacteria in the oral cavity

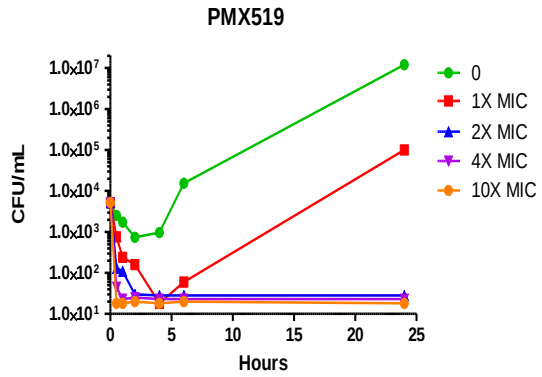
Activity	Compound							
	PMX519	**PMX1408**	**PMX1502**	**PMX1230**	**PMX1570**	**PMX1576**	**PMX1591**	**PMX1625**
C. albicans MIC (µg/ml)	4-8	4	4	8	2	2	2	2
Other Yeast Species MIC (µg/ml)								
C. tropicalis	4	4-8	2-4	4	0.5	0.5	0.5	0.5
C. parapsilosis	8	4-8	2	4-8	2	2	4	4
C. dubliniensis	8	8	4	8-16	2	2	4	4
C. glabrata	16	4	2	8	2	2	4	4
C. krusei	8	32	16	8	2	2	4	4
Cytotoxicity EC50 (µg/ml)								
Mouse NIH3T3	395	221	540	101	68	94	285	332
Human HepG2	>900	322	1097	222	197	181	558	459
OKF6/TERT	>900	332	950	51	235	315	NT	456
Commensal Bacteria MIC (µg/ml)								
Streptococcus salivarius	>64	16	>64	>64	8	8	32	64
Actinomyces viscosus	>64	4	>64	>64	4	4	4	16

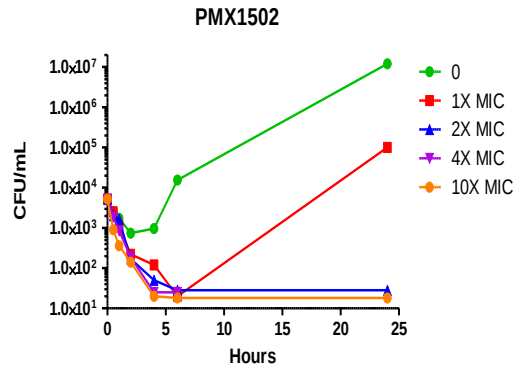
Anti-Candida Leads; Additional *in vitro* Activities

Potent activity against vegetative and 2 day hyphal biofilm cultures

Compound	Anti-*C. albicans* GDH2346 (IC_{50}, µg/ml)	
	Vegetative	Hyphal
PMX70004	4.88	11.04
PMX519	4.93	4.90
PMX1408	4.24	0.71
PMX1502	1.44	2.68
PMX1570	1.09	1.00
PMX1576	1.03	1.40
PMX1591	2.20	ND
PMX1625	2.08	2.22

Cidal activity, rapid killing kinetics evident





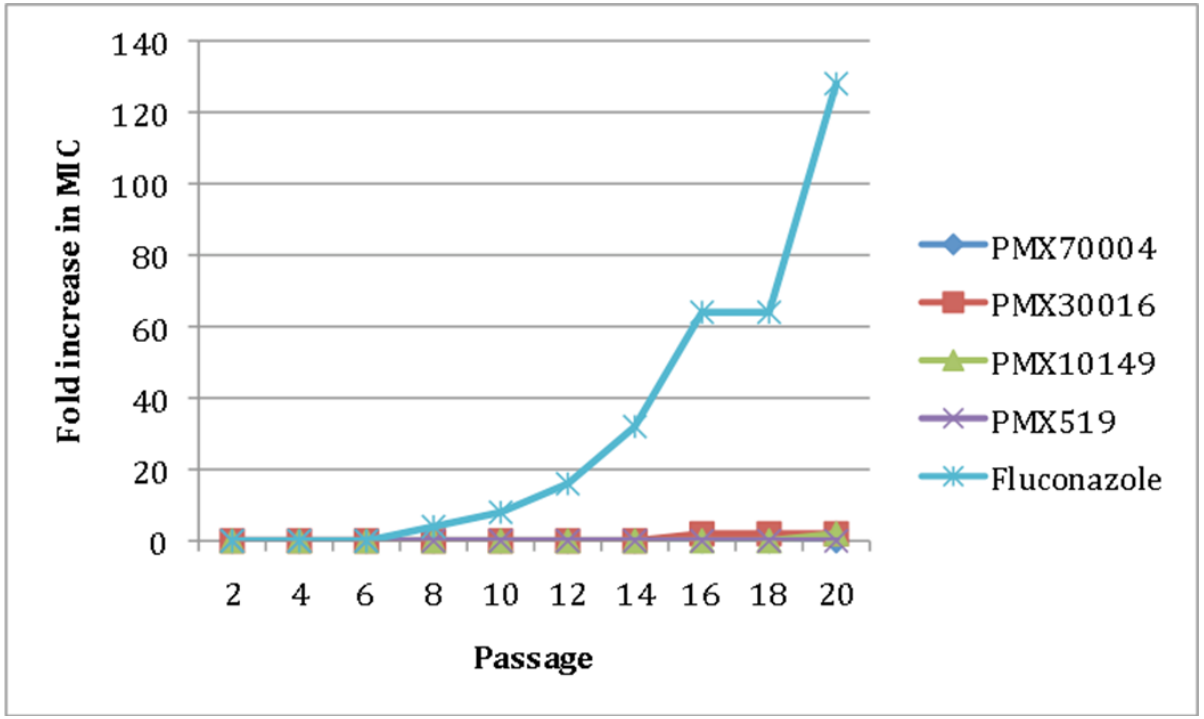
Subset of compounds active in serum

Compound	MIC (µg/ml)			
	PMX1408	PMX1408 +50% HS	PMX1502	PMX1502 +50% HS
C. albicans GDH2346	4	2	4	2
C. dubliniensis	8	2	4	1
C. glabrata	4	1	2	2
HS: human serum				

Serial passage resistance assays with *Candida albicans*.

No evidence for resistance w/ *C. albicans* GDH2346 grown in presence of PMX compounds

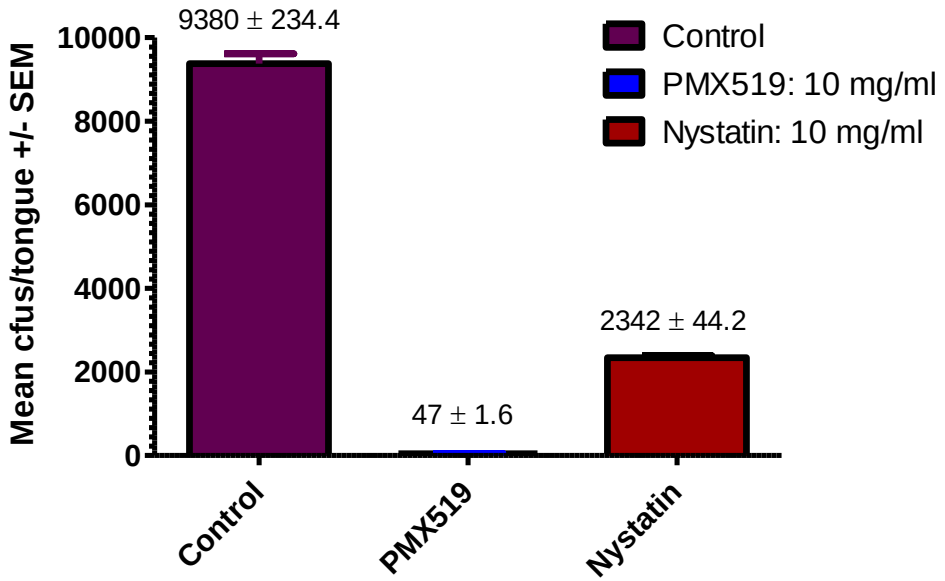
Resistance is readily apparent with Fluconazole



MOA studies measuring influx and efflux of substrates indicate the fungal plasma membrane as a target

Efficacy in a Mouse Model of Oral Candidiasis

3 day oral infection (tongue) w/ *C. albicans* GDH2346
Single topical administration of test agent (10 mg/ml) in 0.1 ml hydrogel on Day 4
Harvest 24 hrs post-treatment, homogenize, quantitate by serial dilution & plating

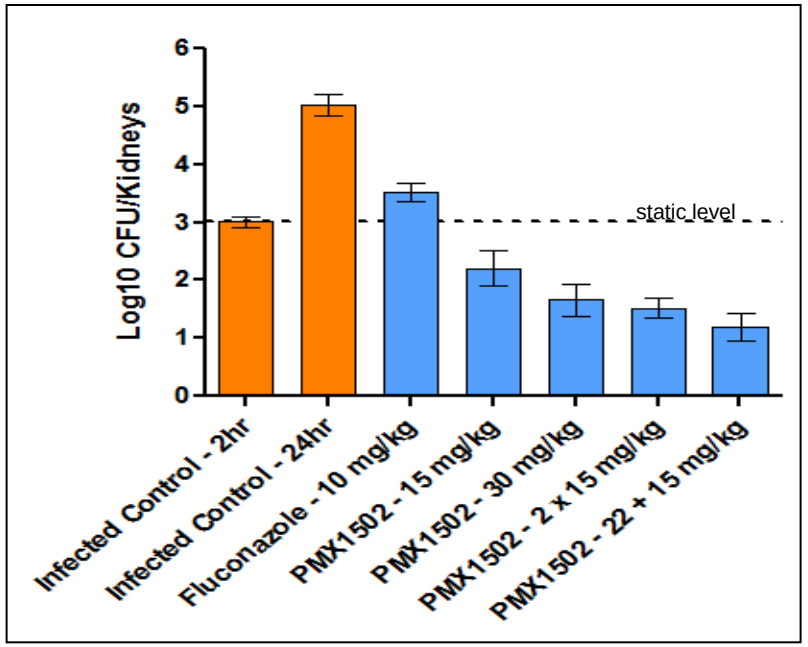


PMX519 nearly sterilizes the infected tongue following a ***single* topical dose and is 50-fold more efficacious than Nystatin**

PMX1502 shows comparable activity to PMX519 in same dosing regimen

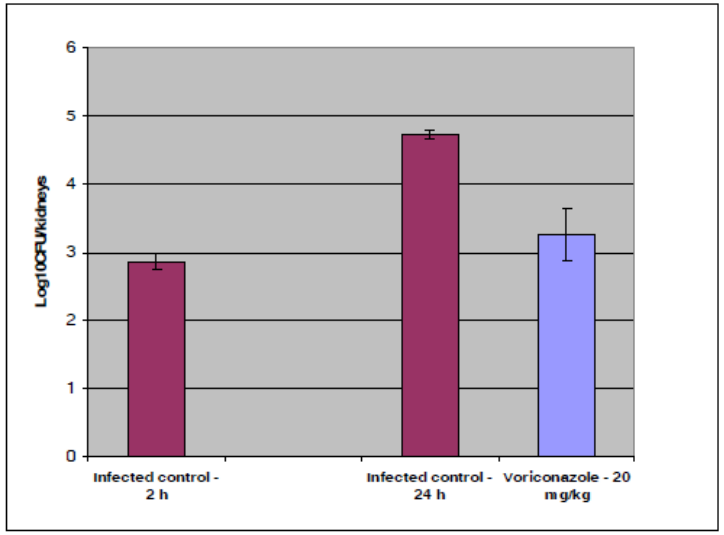
Anti-Fungal Activity:
Efficacy in Mouse Candida Sepsis Model – PMX-1502

- T = 0, neutropenic CD-1 mice were infected IV with 3.4 x 10^4 cfus *C. albicans*
- T = 2 hrs, mice were treated IV with test agent 1x or 2x/day
- T = 24 hrs, kidneys were harvested and quantitated for tissue burden



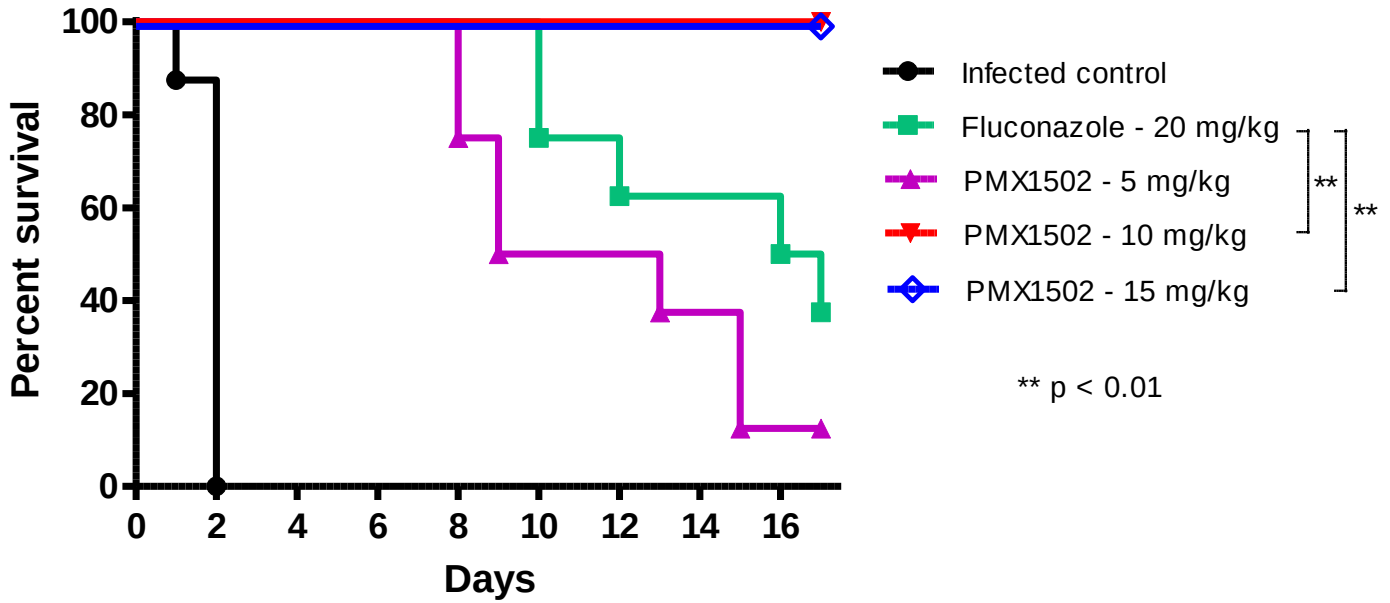
PMX1502 shows cidal activity with a 1.5 log_{10} reductions in tissue burden from treatment onset

Current triazole anti-fungals show a static effect in the model



Disseminated Candidiasis Model; Survival Study

- Neutropenic mice infected IV with *C. albicans* R303 on Day 0
- At 2 hrs post-infection, test agents administered 1x/day for 4 days
  - Fluconazole - oral; PMX1502 - IV
- Survival monitored over next 14 days



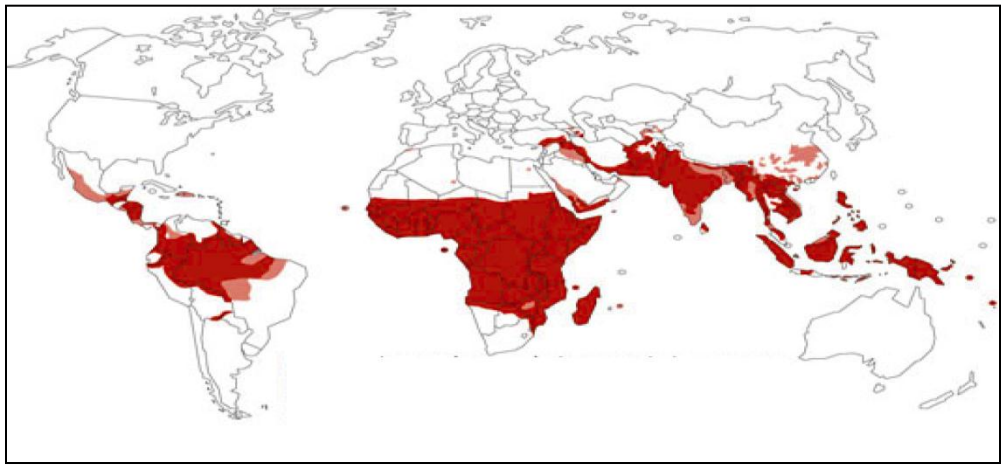
100% survival in 10 and 15 mg/kg PMX1502 groups, no overt toxicity

40% survival in the fluconazole group

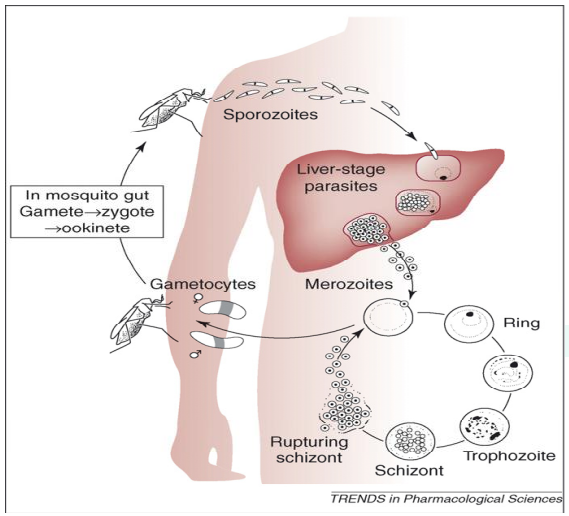
Presentation Topics

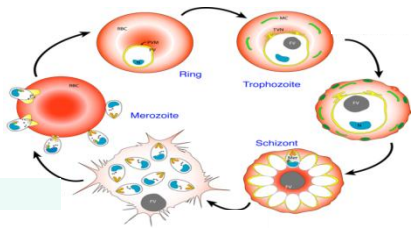
- **Brilacidin; Clinical status**
- **Preclinical development of antimicrobial therapeutics for:**
  - **Gram-negative pathogens**
  - **Fungal pathogens**
  - **Malaria**
- **Oral mucositis; Brilacidin**

Malaria



Resistance to current anti-malarials is common





Disease

- 2-3 billion people exposed
- >500 million infected; 1 million deaths/yr
  - Most children <3-5 yrs old
- *P. falciparum* most dangerous parasitic agent

Current drugs

- Chloroquines and artemisinin
- Mechanisms ill-defined but involve metabolism of hemoglobin/accumulation of toxic by-products
- Resistance widespread vs. chloroquines and rapidly emerging vs. artemisinin

Novel therapies needed to combat resistance

- Small molecules, vaccines
- All small molecules under development target proteins
  - Resistance likely

Approach w/ HDP mimics

- Initially identify preclinical leads by IV administration
- Chemical optimization for oral bioavailability

Anti-malarial *in vitro* lead compounds; Round 1: Library screen and optimized compounds

Initial lead series

- Active vs. CQ-S and CQ-R strains; IC50s < 200 nM
- Low cytotoxicity (EC_{50}) vs mammalian cells: > 500 selectivity index (EC_{50}/IC_{50})
- Negligible hemolysis at 100x IC_{50} concentrations
- Structural diversity
- Low anti-bacterial activity

	CQ-sensitive *P. falciparum*		CQ-resistant *P. falciparum*			Mammalian Cells	
Compound	3D7 IC_{50} (nM)	HB3 IC_{50} (nM)	Dd2 IC_{50} (nM)	7G8 IC_{50} (nM)	K1 IC_{50} (nM)	3T3 EC_{50} (μM)	HepG2 EC_{50} (μM)
Chloroquine	8.7 ± 3.3	8.7 ± 1.0	24.6 ± 2.7	37.0 ± 5.8	47.1 ± 6.0	-	-
PMX611	60 ± 20	26 ± 8	49 ± 14	66 ± 14	57 ± 10	769	830
PMX1207	110 ± 12	104 ± 20	75 ± 11	91 ± 7	83 ± 4	65.1	165
PMX207	153 ± 13	133 ± 13	134 ± 10	104 ± 9	95 ± 13	463	536
PMX496	160 ± 60	150 ± 70	170 ± 70	160 ± 60	170 ± 70	139	80
PMX504	160 ± 60	160 ± 60	118 ± 16	113 ± 15	170 ± 70	322	356
PMX647	160 ± 60	170 ± 70	300 ± 100	300 ± 100	610 ± 66	284	433
PMX835	220 ± 50	200 ± 70	300 ± 100	200 ± 70	200 ± 70	>1000	>1000

Several rounds of chemical optimization have resulted in the identification of additional leads

Chemical optimization for anti-malarial activity

Compound	*P. falciparum* IC_{50} (nM)		Cytotoxicity EC_{50} (uM)		MW (free base)
	3D7 (CQ-S)	Dd2 (CQ-R)	3T3	HepG2	
PMX1572	28.7	24	172	347	602.54
PMX1573	38.3	42.3	65	214	592.57
PMX1625	58.2	110	523	723	407.54
PMX1424	79.5	74.1	>1000	>1000	402.51
PMX1467	87.4	101	74	447	634.67
PMX1560	108.3	95.8	398	702	482.53
PMX1488	129.4	NT	478	>1000	865.91
PMX1533	177.3	NT	63	>1000	912.82
PMX1547	320.3	NT	>1000	>1000	382.47

- **Optimized leads**
  - **Potently active against CQ-S and CQ-R strains**
  - **IC50s in 30 to 130 nM range**
  - **Low cytotoxicity**
  - **Several compounds also have relatively low MWs (400 – 600 D)**
  - **SAR trend: reduced charge density**
- **Trends for activity with smaller and less charged compounds favors oral bioavailability**

Animal Studies; Initial Lead Compounds Safety and Pharmacokinetics

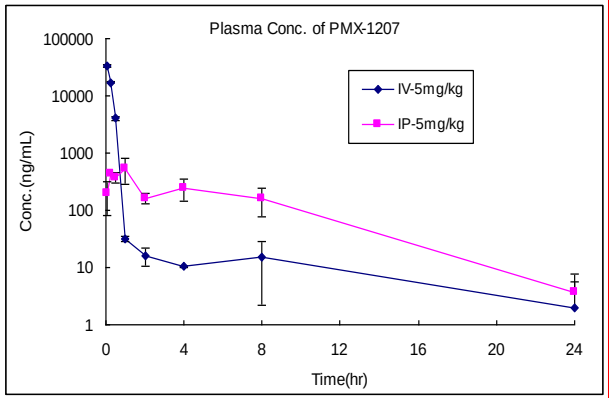
In vivo tolerability

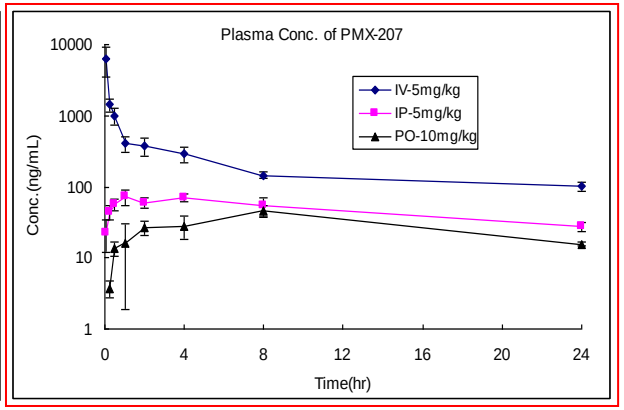
Compound	Maximum Tolerated Dose (MTD; mg/kg)	
	IV (single dose)	IV (repeat dose)
PMX611	< 5	NT
PMX1207	15	5
PMX207	>40	20
PMX496	10	NT
PMX504	< 5	NT
PMX647	30	NT
PMX835	40	NT
NT: Not Tested		

- PMX207 is well tolerated in single and repeat dose MTD studies

Pilot PK results

Compound (5 mg/kg)	Cmax (ng/ml)	$AUC_{0-\infty}$ (ng/mL*h)	$t_{1/2}$ (hrs)
PMX1207	33496	11490	6.75
PMX207	6322	7687	11.7
PMX647	4248	2887	9.37
PMX835	5202	11011	9.52



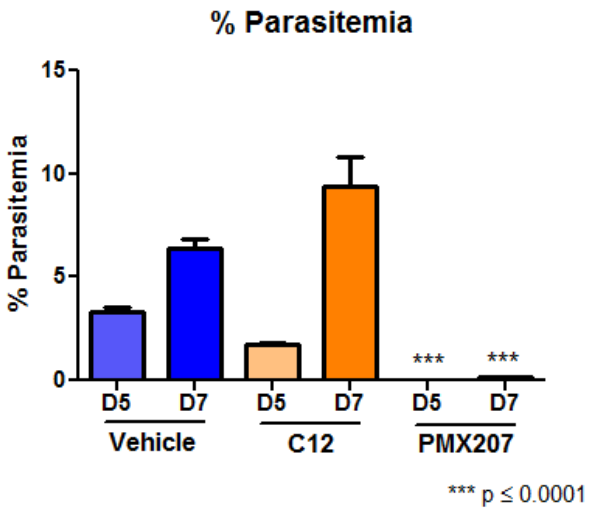


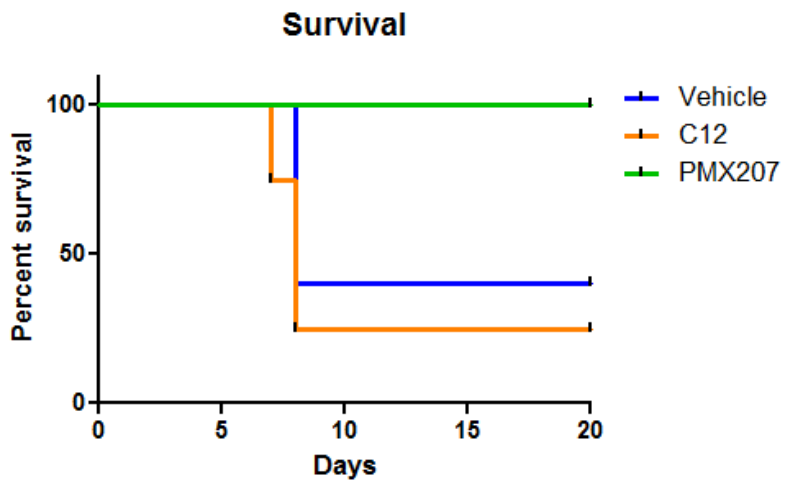
- Highest Cmax and AUC levels reached with PMX1207
- Blood levels of PMX207 exceed IC50s for extended time periods after IV dosing
- PMX207 is 6% orally bioavailable

IV Efficacy Studies in a *P. berghei* ANKA Murine Malaria Model

in collaboration w/ Dr. P. Sinnis (JHU)

- Swiss Webster mice infected IV with $2x10^5$ *P. berghei* ANKA-parasitized erythrocytes
- Mice were infected on day 1 and treated IV on days 1-4, once per day
  - PMX207
  - C12: 12 aa C-terminal fragment of PF4 with anti-*P. falciparum* activity
- Parasitemia was assessed on days 5 and 7 and survival scored through day 20



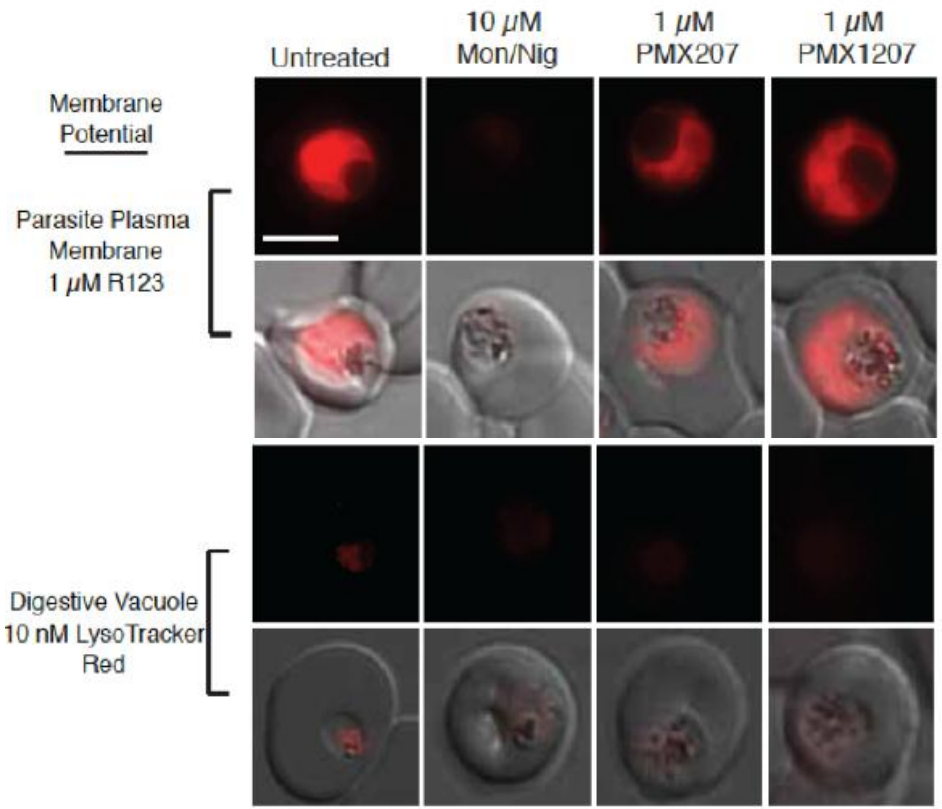


PMX207-treated mice show negligible parasitemia and full survival over 20 day test period

Comparable parasitemia results obtained in Plasmodium yoelli XNL murine model

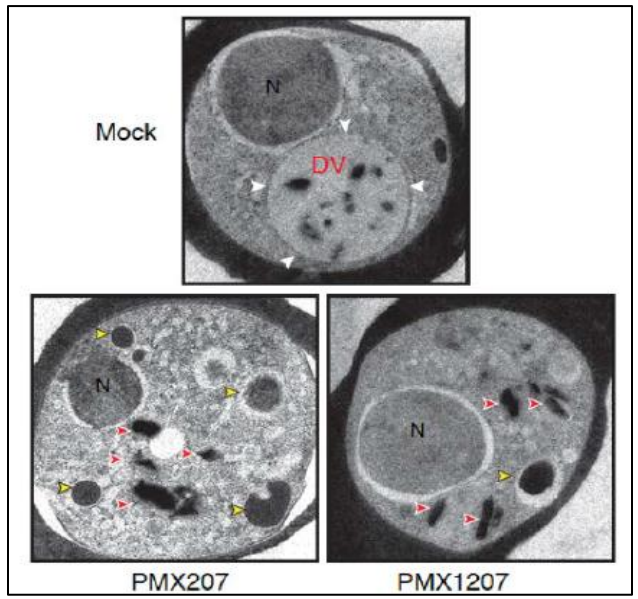
Mechanism of Action; Digestive Vacuole (DV) Lysis

Membrane potential of the *P.f.* plasma membrane remains intact upon 1 hr. treatment w/ PMX1207 and PMX207 but integrity of the digestive vacuole is lost



R123: membrane potential dye rhodamine 123
Mon/Nig: ionophores monensin and nigericin
LysoTrackerRed: acidic vesicle dye

TEM images show complete loss of DV membrane w/ PMX207 and PMX1207 treatment



with dispersal of hemozoin crystals (red arrows) and undigested hemoglobin (yellow arrows)

Presentation Topics

- **Brilacidin; Clinical status**
- **Preclinical development of antimicrobial therapeutics for:**
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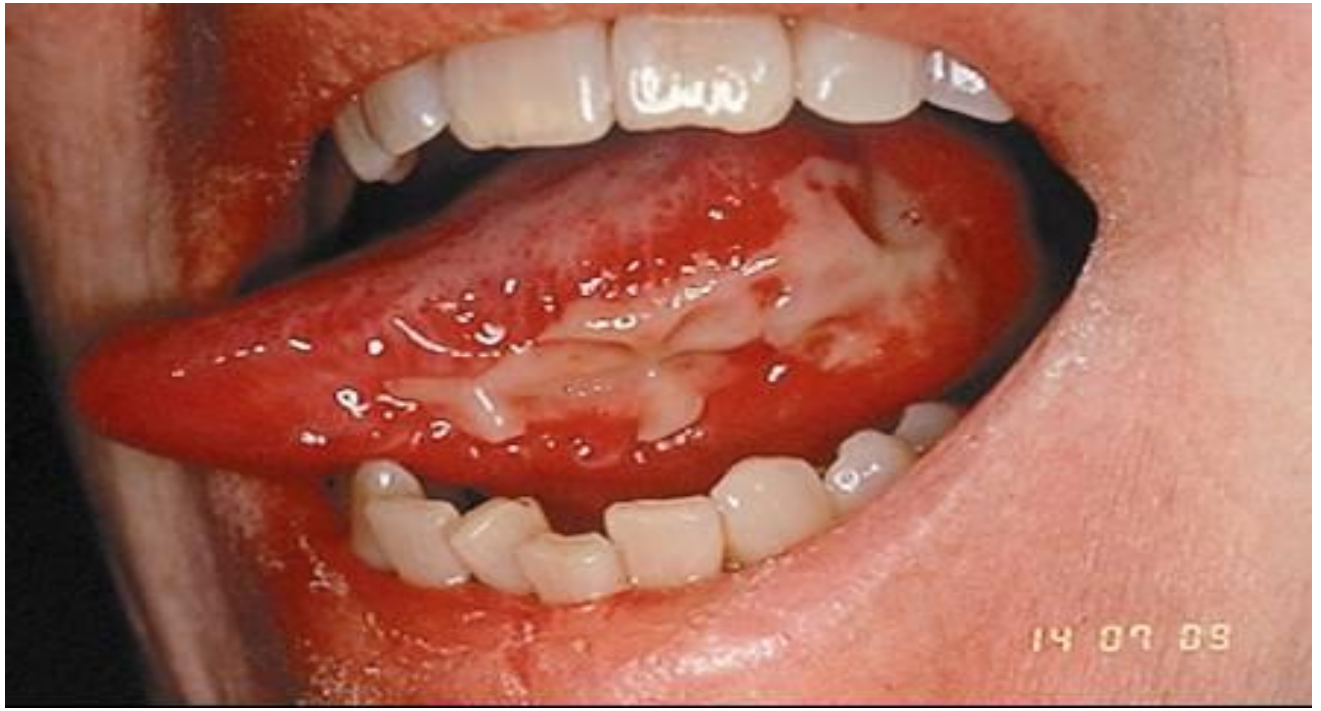
  - **Fungal pathogens**
  - **Malaria**
- **Oral mucositis; Brilacidin**

A Separate Indication for Brilacidin - Oral Mucositis

- **Complication of cancer treatment – radiation & chemotherapies**
- **Can develop into ulceration of the oral mucosa**
- **Debilating; Renders patients unable to speak, eat or tolerate therapy**
- **Can be dose-limiting and lead to reduction/ cessation of the cancer treatment**



Courtesy Dr. Stephen Sonis, Brigham and Women's Hospital, Harvard Medical School

Cancer Oral Mucositis; Pathogenesis

- **Current Model**
  - Continuum of an inflammatory cascade
    - Initiated by DNA damage, ROS production, epithelial cell death
  - Mediated largely by NFKB-dependent pathways
    - Activation of inflammatory cytokines and matrix metalloproteinases
  - Leads to destruction of the oral mucosa and ulceration
- **Bacterial infection; secondary component**
  - Ulcers provide portals for bacterial entry and colonization
  - Exacerbates disease process
  - Septicemia possible
- **HDP mimics**
  - Immune modulatory activities evident in brilacidin series
  - Leverage dual activity into treatment for OM
  - Brilacidin evaluated in hamster acute and fractionated radiation models of OM

Hamster Model for Oral Mucositis; Acute and Fractionated Radiation: Clinical Signs Scoring

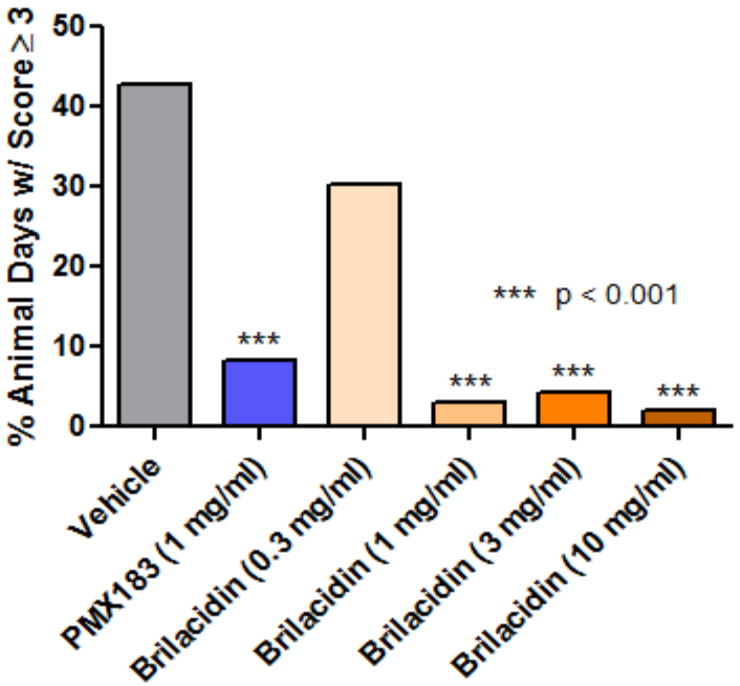
Ulceration: Clinically-significant stage →

Score	Description
0	Pouch completely healthy. No erythema or vasodilation.
1	Light to severe erythema and vasodilation. No erosion of mucosa.
2	Severe erythema and vasodilation. Erosion of superficial aspects of mucosa leaving denuded areas. Decreased stippling of mucosa.
3	Formation of off-white ulcers in one or more places. Ulcers may have a yellow/gray appearance due to pseudomembrane formation. Cumulative size of ulcers should equal about ¼ of the pouch. Severe erythema and vasodilation.
4	Cumulative size of ulcers should equal about ½ of the pouch. Loss of pliability. Severe erythema and vasodilation.
5	Virtually all of pouch is ulcerated. Loss of pliability (pouch can only partially be extracted from mouth.

Brilacidin Significantly Reduces Duration of Mucositis

Oral rinse - Hamster Acute Radiation Model

Duration of Ulcerative Mucositis



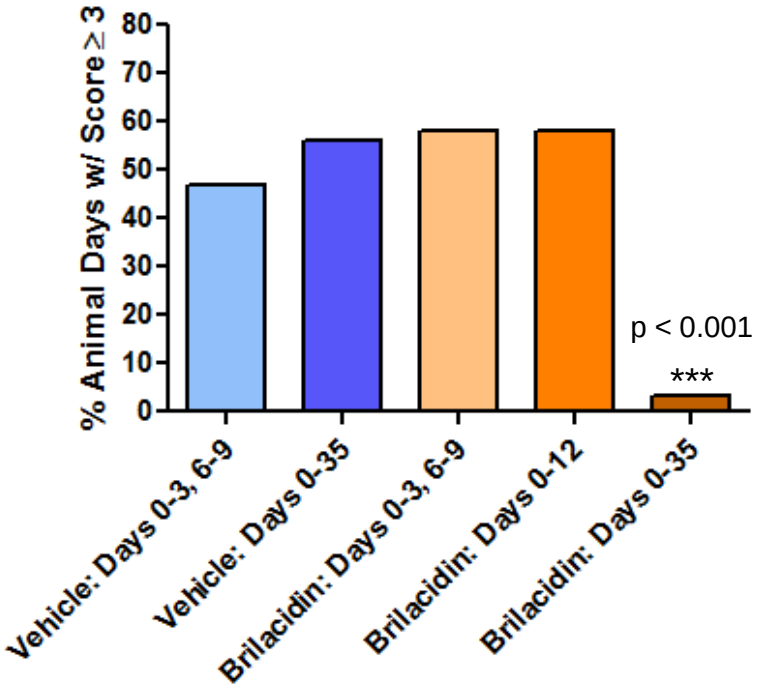
Efficacy was dose-dependent and brilacidin at 1, 3 or 10 mg/mL (TID) reduced the number of animal days of ulcerative oral mucositis to 2 to 4 % from 43% in vehicle-treated animals (p<0.001)

91% - 95% reduction in ulcerative oral mucositis

- Day 0: Animals are given a single radiation dose (40 Gy) to their left buccal cheek pouch
- Day 0 – 20: Test articles are applied topically to the left buccal pouch 3x/day
- Day 6 – 28: Mucositis is evaluated clinically every other day

Hamster Oral Mucositis Model; Duration
Fractionated Radiation Model

Duration of Ulcerative Mucositis



Number of days of mucositis ≥ Grade 3:

Vehicle: 47 - 56%

Brilacidin: 3.3%
(Days 0 – 35)

93% - 94% reduction in ulcerative oral mucositis in a more clinically-relevant and stringent model

Hamsters receive 7.5 Gy radiation dose to left buccal cheek pouch on Days 0,1,2,3,6,7,8 and 9
Treatment begins on Day 0 (TID, 3 mg/ml/dose) and continues daily for the indicated time periods

Cancer Oral Mucositis

- **Critically important unmet clinical need**
  - Common condition in head and neck cancer patients, significant incidence in other populations
  - Only one drug currently available (Kepivance) with a narrow indication
- **Robust efficacy in a widely-used animal model**
- **Preclinical tumor impact study**
  - Brilacidin had no effect on tumor growth or tumor response to chemotherapy or radiation therapy in nude mice
- **Next steps**
  - Mechanism of action and dosing interval studies
  - Oral toxicity; 2 species
- **Clinical trial in H&N patients – begin in H1 2013** *(pending funding)*

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