Cellceutix Corporation
Beverly, MA USA
www.cellceutix.com

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Synthetic Novel Host Defense Protein Mimetics for the Treatment of Gram-Negative Bacterial Infections

Abstract 0169; Presentation 0082; Hall C, 2:30 pm

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Synthetic Novel Host Defense Protein Mimetics for the Treatment of Gram-Negative Bacterial Infections

Author Disclosures

Author	Category	Affiliation	Location
Siya Ram	Employee	Cellceutix Corporation	Beverly, MA, USA
Krishna Menon	Employee	Cellceutix Corporation	Beverly, MA, USA
Richard Scott	Consultant	Fox Chase Chemical Diversity Center	Doylestown, PA, USA
Karima Chafai-Fadela	Employee	Cellceutix Corporation	Beverly, MA, USA
David Brennan	Employee	Cellceutix Corporation	Beverly, MA, USA
William DeGrado	NTD*	University of California at San Diego	San Diego, CA, USA
Damian Weaver	NTD*	Fox Chase Chemical Diversity Center	Doylestown, PA, USA
Katie Freeman	NTD*	Fox Chase Chemical Diversity Center	Doylestown, PA, USA
Gregory Tew	NTD*	University of Massachusetts	Amherst, MA, USA
William Weiss	NTD*	University of North Texas	Ft. Worth, TX, USA
Ashok Kumar	Employee	Cellceutix Corporation	Beverly, MA, USA
Sylvia A. Holden	Employee	Cellceutix Corporation	Beverly, MA, USA

***NTD = Nothing to Disclose**

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Antimicrobial Program; Background

Develop small non-peptidic, fully synthetic mimics of the Host Defense Proteins (HDPs) as systemic and topical agents

- Novel approach for bactericidal activity
- Clinical lead: Brilacidin: Completed two Phase 2 clinical studies for ABSSSI

HDPs are small antimicrobial peptides

- Expression widespread in the animal kingdom
- Produced in skin, mucosal surfaces, neutrophils
- Target microbial membrane

First line of defense against bacterial invasion

- Part of innate immunity
- Maintenance of epithelial barrier function
- Regulate microbiota
- Immuno-modulatory activities – link innate and adaptive immunity

HDP dysfunction implicated in disease

- IBD, atopic dermatitis, acne, otitis media, cystic fibrosis…

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HDP Mimetics: *Lead Clinical Program-Brilacidin*

Phase 1:

- **One single and two multi-dose studies**
- **Pharmacokinetics/Pharmacodynamics**
  - Concentration-dependent killing
  - Highly active vs. *Staph aureus* (MRSA and MSSA)
  - Half-life of ~ 15-23 hours
  - Short-course and single-dose regimens possible

Phase 2:

- **Two studies in ABSSSI (Phase 2a and 2b)**
- **Brilacidin dosed in >300 patients**
  - Multiple dosing regimens explored, including single-dose
  - Safe and generally well tolerated
  - Efficacy in brilacidin regimens comparable to 7 days of daptomycin

Phase 3:

- **Plan to start phase 3 with single-dose regimen**

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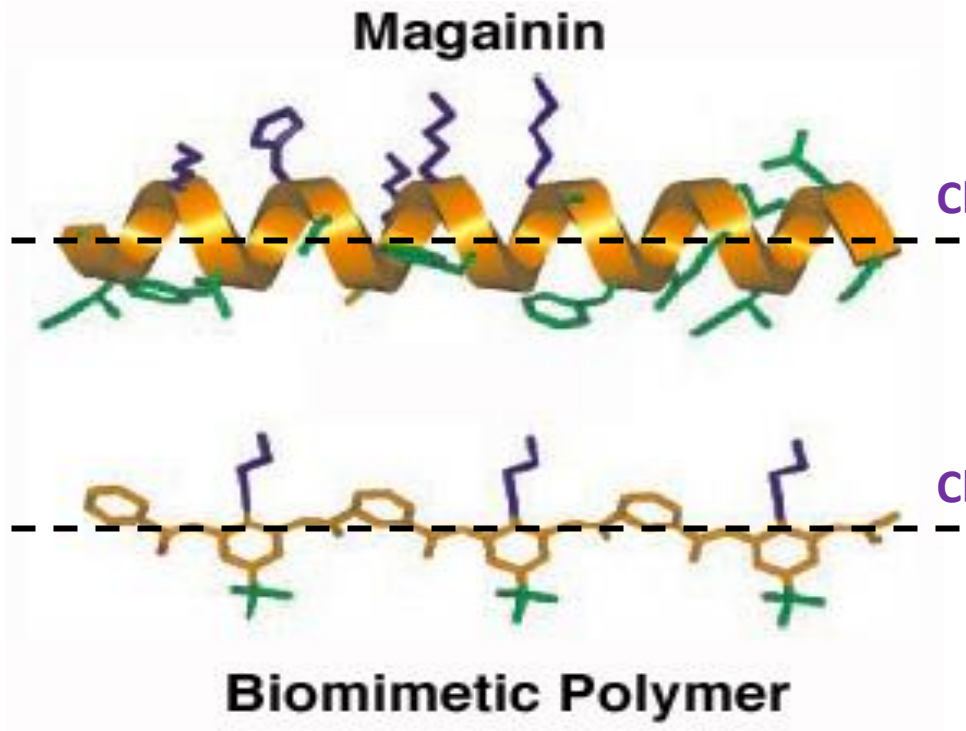
Design Approach

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

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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

Share important antimicrobial properties with HDPs

- Rapidly bactericidal; time-kills 0.5 to 6 hrs
- Low potential for resistant development; 20 serial passage assays and fsr < 10^{-11}

Metabolically stable and active *in vivo*

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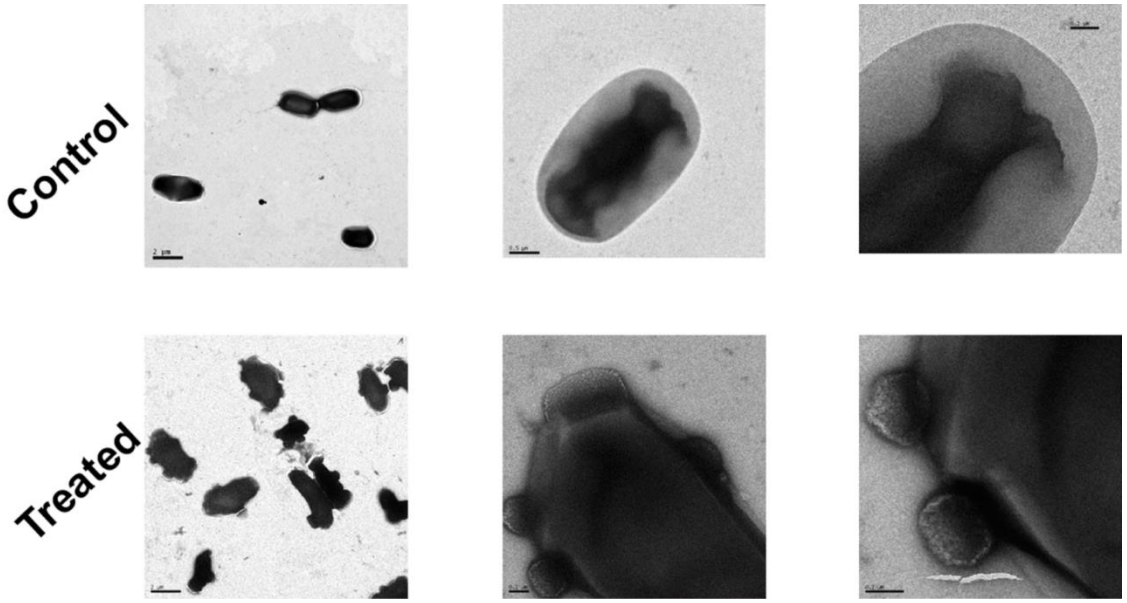
Mechanism of Action: Membrane Target

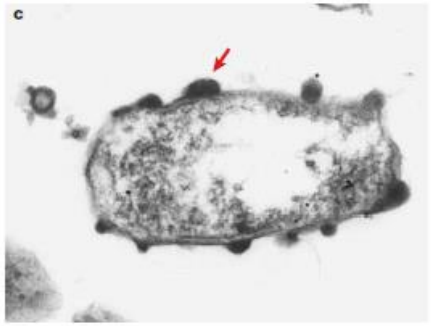
Membrane activity in Gram-positive and Gram-negative organisms supported by

- **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.

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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

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

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Carbepenem-Resistant Enterobacteriaceae (CRE)

Urgent Public Health Threat in U.S, Europe, and the World

CDC, Antibiotic Resistance Threats in The United States, 2013

International dissemination of *Klebsiella pneumoniae* carbapenemase (KPC)–producing *Enterobacteriaceae*.

Clinical Infectious Diseases 2011;53(1):60–67

Expansion of healthcare-associated carbapenem-non-susceptible *Enterobacteriaceae* in Europe: epidemiological scale and stages by country, as of July 2010

Country	Stage	Epidemiological scale	Documented introduction from abroad	Dominant class	Underreporting
Greece	5	Endemic	Yes	KPC/VIM	
Israel[a]				KPC	
Italy	4	Interregional spread	Yes	KPC	
Poland					Likely
France	3	Regional spread	Yes	KPC	
Germany				OXA-48/VIM	Likely
Hungary				KPC	Likely
Belgium	2b	Independent hospital outbreaks	Yes	VIM	Likely
Spain				KPC/VIM/IMP	Likely
England and Wales				NDM	

Eurosurveillance: http://www.eurosurveillance.org

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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
100	AR	0.78 – 1.56	1.56 – 3.13	128	145
183	AA	0.78 – 3.13	1.56 – 3.13	139	227
223	AA	0.78 – 3.13	0.78 – 3.13	178	480
brilacidin	AA	0.78 – 1.56	0.78 – 3.13	727	684
247	AA	1.56	0.78 – 3.13	27	71
519	AA	1.56	0.78 – 3.13	430	1000
843	AA	1.56	1.56 – 3.13	79	131
1091	AA	1.56 – 3.13	1.56 – 3.13	389	724
1099	AR	1.56	1.56	57	106
1278	TA	0.39 – 0.78	0.78	192	>1000
1363	AA	0.78 – 1.56	1.56 – 3.13	422	262
1405	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

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Susceptibility of Drug-R Pathogens;

ndm-1 *K. pneumoniae*

Compound	MICs (µg/ml) vs. *K. pneumoniae* strains		
	ndm-1 (BAA 2146)	2 ATCC strains (non-ndm-1)	5 clinical isolates (non-ndm-1)
868	0.78	0.39	ND
1090	0.78	0.78	ND
100	1.56	0.39	1 - 4
223	1.56	0.78	1 - 2
225	1.56	1.56	2 - 4
183	3.13	0.39	1 - 2
668	3.13	1.56	4
519	3.13	1.56	2
brilacidin	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

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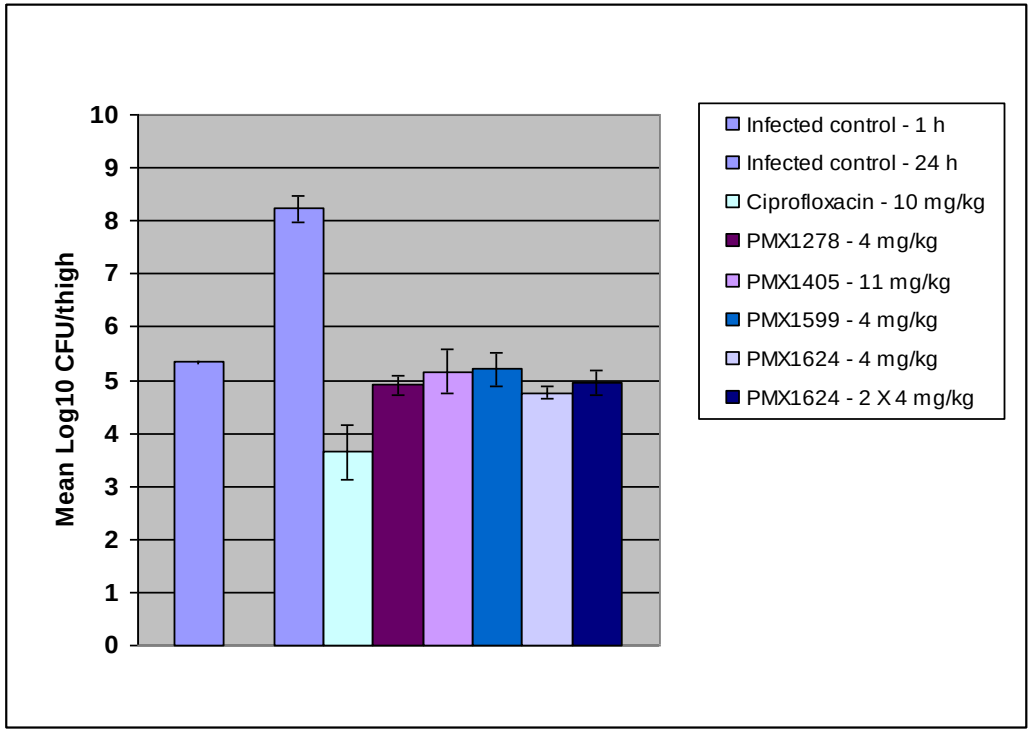
Animal Efficacy vs. *E. coli*; Mouse Thigh Burden model

Screening model for *in vivo* efficacy: Mouse thigh burden

Promising new series showing robust activity identified; the triaryls

PMX1241 and 3 other triaryls are highly active vs. *E. coli* in the TBM



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

Studies conducted at Ricerca Biosciences, Concord, OH

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Activity profiles of lead compounds active vs. *K. pneumoniae*

Cmpd	MIC (µg/ml)											Cytotoxicity (EC_{50}, µM)		FW
	EC (25922)	EC (25922) +40%ms	SA (27660)	SA (27660) +40%ms	EF (29212)	PA (10145)	KP (13883)	KP (13883) +40%ms	AB (17978)	AB (BAA-1605)	AB (19606)	HepG2	NIH3T3	
1278	0.78	12.5	0.39	3.13	6.25	25	0.78	12.5	25	50	50	192	>1000	773
1741	0.78	3.13	0.39	0.78	3.13	25	0.2	6.26	50	50	>50	463	697	909
1807	0.39	0.78	0.39	0.39	1.56	>50	0.39	1.56	25	>50	>50	181	472	859

EC: *E. coli* 25922; SA: *S. aureus* 27660; EF: *E. faecalis* 29212; PA: *P. aeruginosa* 10145; KP: *K. pneumoniae* 13883; AB: *A. baumannii* 17978, BAA-1605, 19606; HepG2: human transformed liver cells; NIH3T3: mouse 3T3 fibroblasts; FW: Formula molecular weight (salt form); ms: mouse serum; NT: Not Tested; All MIC assays were conducted according to Hancock modifications of CLSI guidelines for cationic compounds. Cytotoxicity was measured in XTT assays for metabolic activity (Promega).

Active vs. *E.coli*, *K. pneumoniae* and Gram-positives in absence and presence of serum
Little activity vs. *A. baumannii* or *P. aeruginosa*
Good cytotoxicity profile vs. mammalian cells (> 100 fold selectivity)

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Activity Lead Compounds vs. MDR *K. pneumoniae* strains

Cmpd	MIC (µg/ml)											
	Kpn UNT180-1 (KPC isolate)			Kpn UNT153-1 (KPC isolate)			Kpn UNT024-1 (Drug-susceptible strain)			Kpn UNT127-1 (ndm-1 isolate)		
	MHB	MHB + 40% MS	MHB + 5% MS	MHB	MHB + 40% MS	MHB + 5% MS	MHB	MHB + 40% MS	MHB + 5% MS	MHB	MHB + 40% MS	MHB + 5% MS
1807	2	2	2	2	2	4	1	2	2	1	4	2
1741	2	2	2	4	8	4	2	2	8	4	4	8
1278	4	16	2	> 16	> 16	> 16	1	4	4	> 16	2	4

UNT180-1: KPC producer; UNT153-1: KPC producer; UNT024-1: ATCC43816; UNT127-1: ndm-1 producer. Kpn: Klebsiella pneumoniae. MHB: Mueller Hinton broth. ms: mouse serum. All MIC assays were conducted under CLSI guidelines.

-Compounds are active vs. Drug-S and CRE organisms

-Serum has little impact on activity of 1807 and 1741

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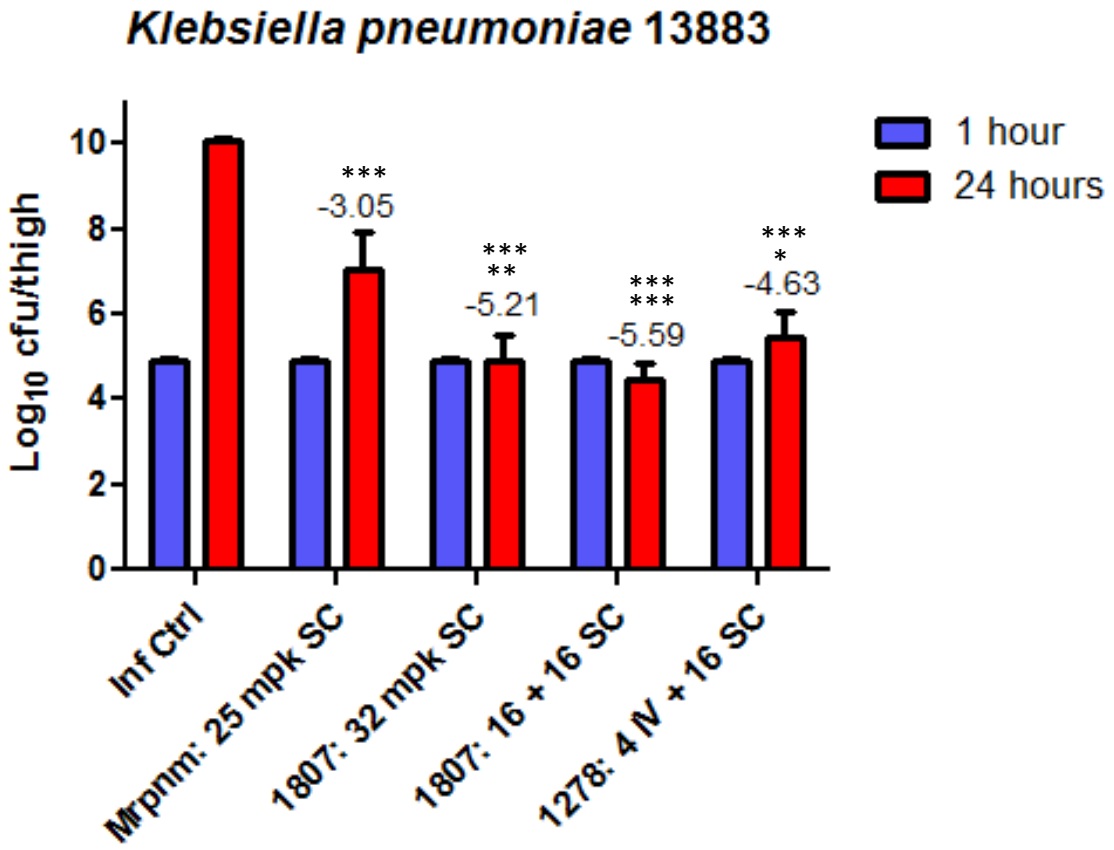
Efficacy in Mouse Thigh Burden Model; IV and SC Dosing

Model:

- T = 0; neutropenic CD-1 mice are infected with 1.0 x 10^5 cfus Kp in thigh muscles
- T = 2 hr: test agent administered SC or IV 1x or 2x/day
- T = 26 hrs: Thighs are harvested for quantitation of tissue burden



Studies conducted in collaboration with W. Weiss, UNT Health Science Center

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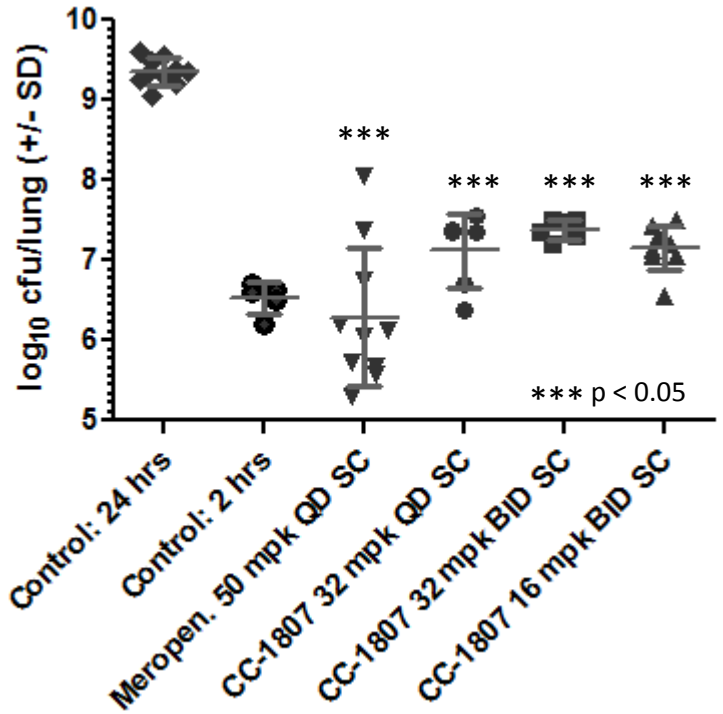
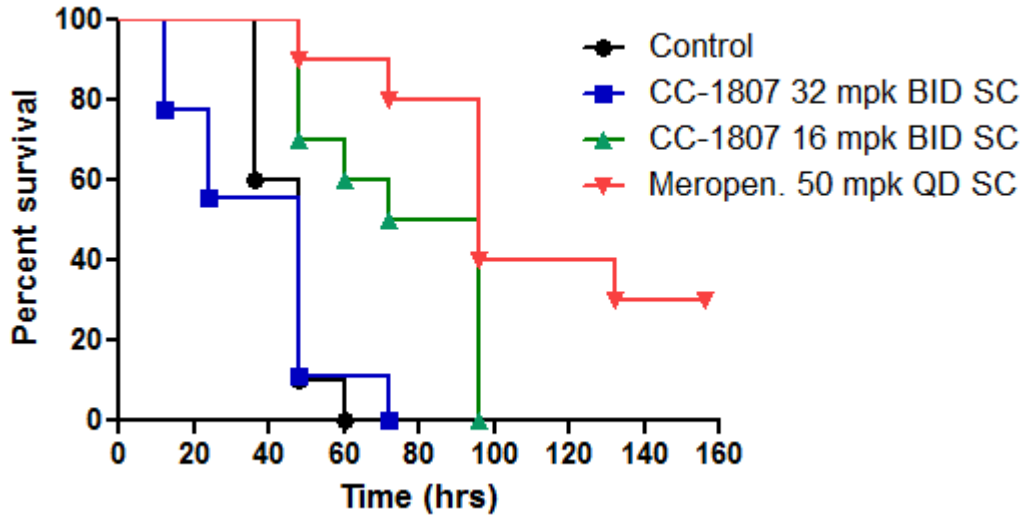
Mouse Lung Infection Model; CC-1807

T = 0 hrs: Infect intranasally w/ 6.53 $\log_{10}$ CFU *K. pneumoniae* 43816
T = 2 hrs: Treat with test agent SC; 1 day for burden and 4 days for survival
T = 24 hrs: Harvest lung tissue for burden measurements (n = 10/group)
T = 0 – 160 hrs: Score for survival (n = 10/group)





Significant reduction in lung burden at all doses of CC-1807
Partial survival effect; toxicity evident at highest dose
Dose optimization in progress

Studies conducted in collaboration with W. Weiss, UNT Health Science Center

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Clinical Isolate Screen w/ CC-1807 vs. *Enterobacteriaceae* spp.

Activity vs. recent collection of clinical isolates

Organism (20 isolates)	MIC_{90} (µg/ml, CLSI)			
	CC-1807	Levofloxacin	Gentamicin	Meropenem
Escherichia coli	0.25	>4	32	0.015
Klebsiella pneumonia	1	>4	1	4
Enterobacter spp.	1	0.5	0.5	0.25

Activity vs. MDR* *Enterobacteriaceae* clinical isolates

Organism	MIC_{90} (µg/ml, CLSI)			
	CC-1807	Levofloxacin	Gentamicin	Meropenem
Escherichia coli (4 isolates)				
MIC range	≤0.06 – 0.12	>4	1 - >64	0.008 - 0.03
Klebsiella pneumonia (8 isolates)				
MIC range	0.25 – 1	>4	≤0.06 – >64	0.06 - >4
Enterobacter cloacae (3 isolates)				
MIC range	0.25 - 2	2 - >4	0.25 – >64	0.25 - >4
MDR *Enterobacteriaceae* (15 isolates)				
MIC range	**≤0.06 – 2**	**2 - >4**	**≤0.06 – >64**	**0.008 - >4**
MIC_{50}	**0.5**	**>4**	**64**	**0.5**
MIC_{90}	**2**	**>4**	**>64**	**>4**

*** resistant to ≥ 3 antibiotic classes, including carbapenems**

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Summary
HDP Mimics for Gram-Negative Infections
Including CRE Strains

- Gram-negative activity evident in several structural series of small non-peptidic mimetics of host defense proteins
- 2 series show low cytotoxicity, favorable PK properties and robust efficacy *in vitro* and *in vivo* against *Enterobacteriaceae* organisms
- CC-1807 is potently active against clinical isolates of *E. coli, K. pneumoniae* and *E. cloacae*, including MDR CRE strains
- Additional preclinical efficacy studies with CC-1807 are in progress
  - Dose optimization in lung infection models
  - UTI and bacteremia
- CC-1807 is negative at 30 µM in *in vitro* manual patch clamp hERG assay
- LPS-neutralizing activity also evident in the CC-1807 and other series
- Chemical optimization of CC-1807 and additional analogs is continuing
  - Expand coverage to *Pseudomonas* and *Acinetobacter* spp.

Other Cellceutix Presentations
ECCMID 2015

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ECMIID 2015
Copenhagen, Denmark
25 – 28 April 2015

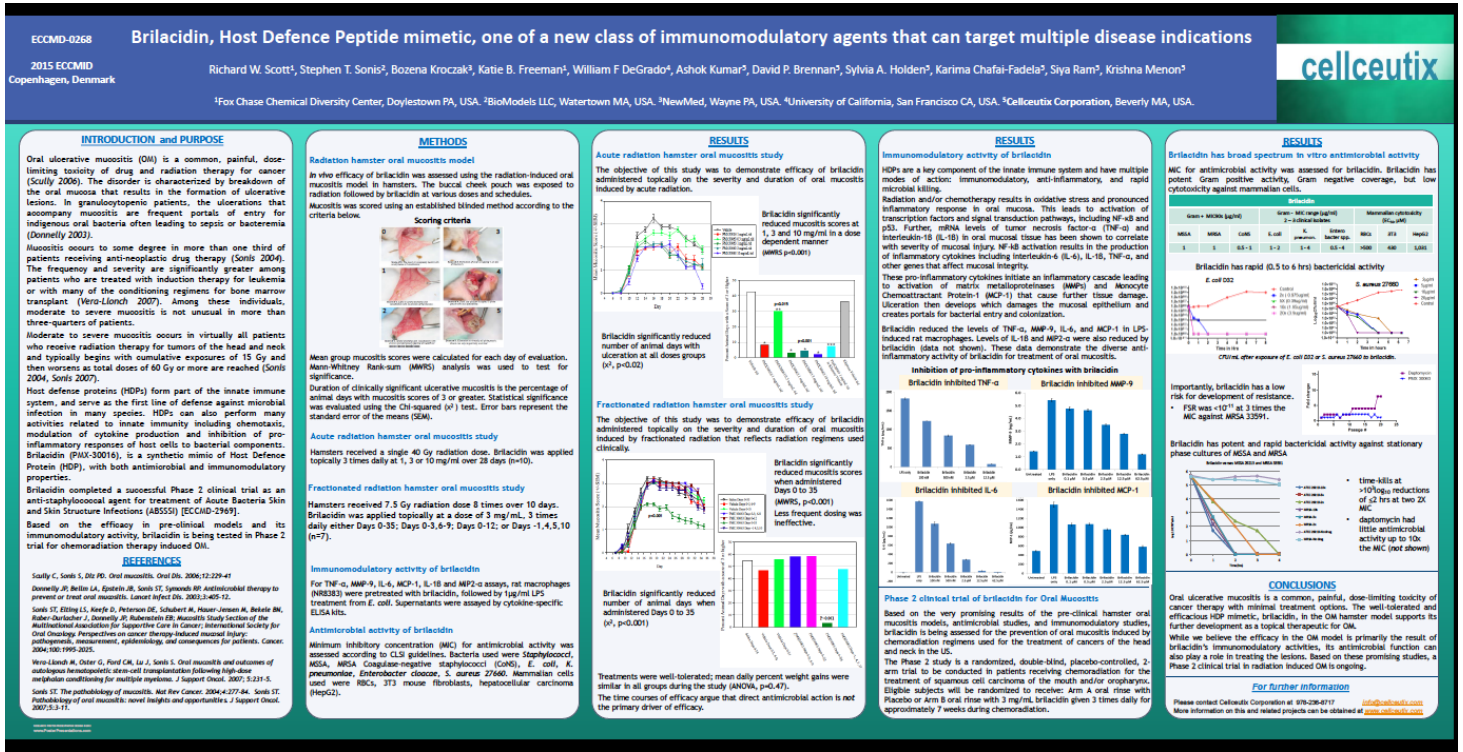
A Randomized, Double-Blind Study Comparing Single-Dose and Short-Course Brilacidin to Daptomycin in the Treatment of Acute Bacterial Skin & Skin Structure Infections (ABSSSI)

Presentation 2969, Hall J, 4:00pm

April 27, 2015

Oral Presentation #0195

e-Poster #EV0201



ECCMD-0268
2015 ECCMID
Copenhagen, Denmark

Brilacidin, Host Defence Peptide mimetic, one of a new class of immunomodulatory agents that can target multiple disease indications

Richard W. Scott[1], Stephen T. Sonis[2], Bozena Kroczak[3], Katie B. Freeman[1], William F DeGrado[4], Ashok Kumar[5], David P. Brennan[5], Sylvia A. Holden[2], Karima Chafai-Fadela[3], Siya Ram[3], Krishna Menon[3]

[1]Fox Chase Chemical Diversity Center, Doylestown PA, USA. [2]BioModels LLC, Watertown MA, USA. [3]NewMed, Wayne PA, USA. [4]University of California, San Francisco CA, USA. [5]Cellceutix Corporation, Beverly MA, USA.

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INTRODUCTION and PURPOSE

Oral ulcerative mucositis (OM) is a common, painful, dose-limiting toxicity of drug and radiation therapy for cancer (*Scully 2006*). The disorder is characterized by breakdown of the oral mucosa that results in the formation of ulcerative lesions. In granulocytopenic patients, the ulcerations that accompany mucositis are frequent portals of entry for indigenous oral bacteria often leading to sepsis or bacteremia (*Donnelly 2003*).

Mucositis occurs to some degree in more than one third of patients receiving anti-neoplastic drug therapy (*Sonis 2004*). The frequency and severity are significantly greater among patients who are treated with induction therapy for leukemia or with many of the conditioning regimens for bone marrow transplant (*Vera-Llonch 2007*). Among these individuals, moderate to severe mucositis is not unusual in more than three-quarters of patients.

Moderate to severe mucositis occurs in virtually all patients who receive radiation therapy for tumors of the head and neck and typically begins with cumulative exposures of 15 Gy and then worsens as total doses of 60 Gy or more are reached (*Sonis 2004, Sonis 2007*).

Host defense proteins (HDPs) form part of the innate immune system, and serve as the first line of defense against microbial infection in many species. HDPs can also perform many activities related to innate immunity including chemotaxis, modulation of cytokine production and inhibition of pro-inflammatory responses of host cells to bacterial components. Brilacidin (PMX-30016), is a synthetic mimic of Host Defence Protein (HDP), with both antimicrobial and immunomodulatory properties.

Brilacidin completed a successful Phase 2 clinical trial as an anti-staphylococcal agent for treatment of Acute Bacteria Skin and Skin Structure Infections (ABSSSI) [ECCMD-2969].

Based on the efficacy in pre-clinical models and its immunomodulatory activity, brilacidin is being tested in Phase 2 trial for chemoradiation therapy induced OM.

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METHODS

Radiation hamster oral mucositis model

In vivo efficacy of brilacidin was assessed using the radiation-induced oral mucositis model in hamsters. The buccal cheek pouch was exposed to radiation followed by brilacidin at various doses and schedules.

Mucositis was scored using an established blinded method according to the criteria below.

Scoring criteria

Mean group mucositis scores were calculated for each day of evaluation. Mann-Whitney Rank-sum (MWRS) analysis was used to test for significance.

Duration of clinically significant ulcerative mucositis is the percentage of animal days with mucositis scores of 3 or greater. Statistical significance was evaluated using the Chi-squared (x^2) test. Error bars represent the standard error of the means (SEM).

Acute radiation hamster oral mucositis study

Hamsters received a single 40 Gy radiation dose. Brilacidin was applied topically 3 times daily at 1, 3 or 10 mg/ml over 28 days (n=10).

Fractionated radiation hamster oral mucositis study

Hamsters received 7.5 Gy radiation dose 8 times over 10 days. Brilacidin was applied topically at a dose of 3 mg/mL, 3 times daily either Days 0-35; Days 0-3.6-9; Days 0-12; or Days -1.4.5.10 (n=7).

Immunomodulatory activity of brilacidin

For TNF-α, MMP-9, IL-6, MCP-1, IL-1B and MIP2-α assays, rat macrophages (NR8383) were pretreated with brilacidin, followed by 1μg/ml LPS treatment from *E. coli*. Supernatants were assayed by cytokine-specific ELISA kits.

Antimicrobial activity of brilacidin

Minimum inhibitory concentration (MIC) for antimicrobial activity was assessed according to CLSI guidelines. Bacteria used were *Staphylococci*, MSSA, MRSA Coagulase-negative staphylococci (CoNS), *E. coli*, *K. pneumoniae*, *Enterobacter cloacae*, *S. aureus* 27660. Mammalian cells used were RBCs, 3T3 mouse fibroblasts, hepatocellular carcinoma (HepG2).

RESULTS

Acute radiation hamster oral mucositis study

The objective of this study was to demonstrate efficacy of brilacidin administered topically on the severity and duration of oral mucositis induced by acute radiation.

Brilacidin significantly reduced mucositis scores at 1, 3 and 10 mg/ml in a dose dependent manner (MWRS p=0.001)

Brilacidin significantly reduced number of animal days with ulceration at all doses groups (x^2, p<0.02)

Fractionated radiation hamster oral mucositis study

The objective of this study was to demonstrate efficacy of brilacidin administered topically on the severity and duration of oral mucositis induced by fractionated radiation that reflects radiation regimens used clinically.

Brilacidin significantly reduced mucositis scores when administered Days 0 to 35 (MWRS, p<0.001)

Less frequent dosing was ineffective.

Brilacidin significantly reduced number of animal days when administered Days 0 to 35 (x^2, p<0.001)

Treatments were well-tolerated; mean daily percent weight gains were similar in all groups during the study (ANOVA, p=0.47).

The time courses of efficacy argue that direct antimicrobial action is *not* the primary driver of efficacy.

RESULTS

Immunomodulatory activity of brilacidin

HDPs are a key component of the innate immune system and have multiple modes of action: immunomodulatory, anti-inflammatory, and rapid microbial killing.

Radiation and/or chemotherapy results in oxidative stress and pronounced inflammatory response in oral mucosa. This leads to activation of transcription factors and signal transduction pathways, including NF-κB and p53. Further, mRNA levels of tumor necrosis factor-α (TNF-α) and interleukin-1B (IL-1B) in oral mucosal tissue has been shown to correlate with severity of mucosal injury. NF-kB activation results in the production of inflammatory cytokines including interleukin-6 (IL-6), IL-1B, TNF-α, and other genes that affect mucosal integrity.

These pro-inflammatory cytokines initiate an inflammatory cascade leading to activation of matrix metalloproteinases (MMPs) and Monocyte Chemoattractant Protein-1 (MCP-1) that cause further tissue damage. Ulceration then develops which damages the mucosal epithelium and creates portals for bacterial entry and colonization.

Brilacidin reduced the levels of TNF-α, MMP-9, IL-6, and MCP-1 in LPS-induced rat macrophages. Levels of IL-1B and MIP2-α were also reduced by brilacidin (data not shown). These data demonstrate the diverse anti-inflammatory activity of brilacidin for treatment of oral mucositis.

Inhibition of pro-inflammatory cytokines with brilacidin

Phase 2 clinical trial of brilacidin for Oral Mucositis

Based on the very promising results of the pre-clinical hamster oral mucositis models, antimicrobial studies, and immunomodulatory studies, brilacidin is being assessed for the prevention of oral mucositis induced by chemoradiation regimens used for the treatment of cancers of the head and neck in the US.

The Phase 2 study is a randomized, double-blind, placebo-controlled, 2-arm trial to be conducted in patients receiving chemoradiation for the treatment of squamous cell carcinoma of the mouth and/or oropharynx. Eligible subjects will be randomized to receive: Arm A oral rinse with Placebo or Arm B oral rinse with 3 mg/mL brilacidin given 3 times daily for approximately 7 weeks during chemoradiation.

RESULTS

Brilacidin has broad spectrum in vitro antimicrobial activity

MIC for antimicrobial activity was assessed for brilacidin. Brilacidin has potent Gram positive activity, Gram negative coverage, but low cytotoxicity against mammalian cells.

Brilacidin								
Gram + MIC90s (μg/ml)			Gram – MIC range (μg/ml) 1 – 3 clinical isolates			Mammalian cytotoxicity (IC_{50},μM)		
MSSA	MRSA	CoNS	E.coli	K. pneumon.	Entero bacter spp.	RBCs	3T3	HepG2
1	1	0.5 – 1	1 – 2	1 – 4	0.5 – 4	>500	630	1,041

Brilacidin has rapid (0.5 to 6 hrs) bactericidal activity

Importantly, brilacidin has a low risk for development of resistance.

- FSR was <10^{-11} at 3 times the MIC against MRSA 33591.

Brilacidin has potent and rapid bactericidal activity against stationary phase cultures of MSSA and MRSA

- time-kills at >$10^{3}$$log_{10}$ reductions of ≥2 hrs at two 2X MIC
- daptomycin had little antimicrobial activity up to 10x the MIC (*not shown*)

CONCLUSIONS

Oral ulcerative mucositis is a common, painful, dose-limiting toxicity of cancer therapy with minimal treatment options. The well-tolerated and efficacious HDP mimetic, brilacidin, in the OM hamster model supports its further development as a topical therapeutic for OM.

While we believe the efficacy in the OM model is primarily the result of brilacidin's immunomodulatory activities, its antimicrobial function can also play a role in treating the lesions. Based on these promising studies, a Phase 2 clinical trial in radiation induced OM is ongoing.

For further information

Please contact Cellceutix Corporation at: 978-236-6717
More information on this and related projects can be obtained at www.cellceutix.com