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

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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 (PMX30063), 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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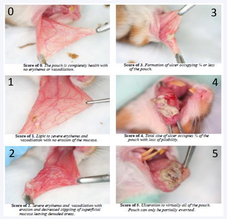
METHODS

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 for 45 min, followed by 1µg/ml LPS treatment from *E. coli* for 8 hours Supernatants were assayed by cytokine-specific ELISA kits.

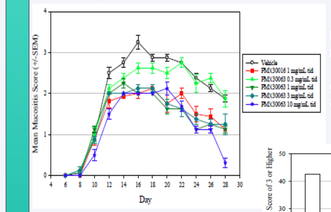
Antimicrobial activity of brilacidin

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).

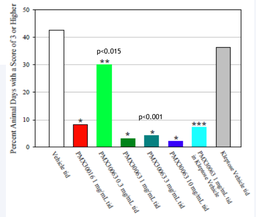
RESULTS

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 (Scott 2012).



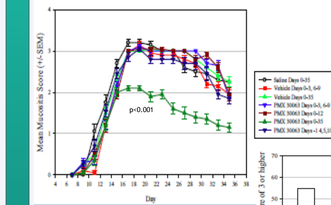
Brilacidin significantly reduced mucositis scores at 1, 3 and 10 mg/ml in a dose dependent manner (MWRS p<0.001)



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

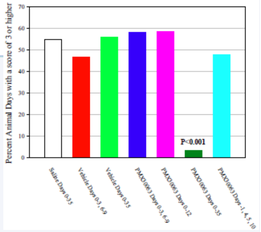
Fractionated radiation hamster oral mucositis study

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 (Scott 2012).



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 (Scott 2012).

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.

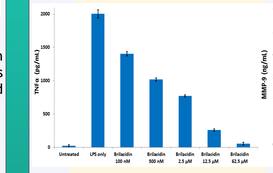
Radiation and/or chemotherapy results in oxidative stress and pronounced inflammatory response in oral mucosa. This leads to activation of transcription factors and signal transduction pathways, including NF-κB and p53. Further, mRNA levels of tumor necrosis factor-α (TNF-α) and interleukin-1B (IL-1B) 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.

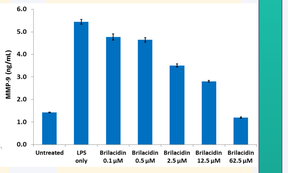
Brilacidin reduced the levels of TNF-α, MMP-9, IL-6, and MCP-1 in LPS-induced rat macrophages. Levels of IL-1B and MIP2-α were also reduced by brilacidin (*data not shown*). These data demonstrate the diverse anti-inflammatory activity of brilacidin for treatment of oral mucositis.

Inhibition of pro-inflammatory cytokines with brilacidin

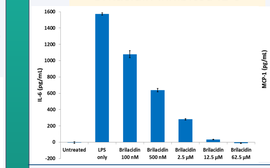
Brilacidin inhibited TNF-α



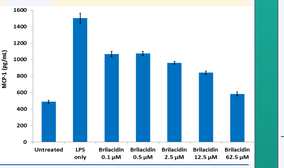
Brilacidin inhibited MMP-9



Brilacidin inhibited IL-6



Brilacidin inhibited MCP-1



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.

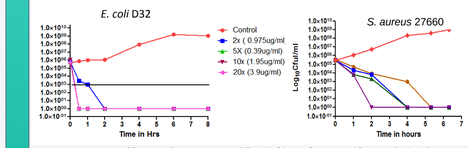
RESULTS

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) 2 – 3 clinical isolates			Mammalian cytotoxicity (EC_{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	430	1,031

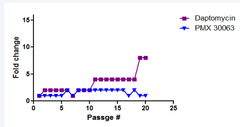
Brilacidin has rapid (0.5 to 6 hrs) bactericidal activity



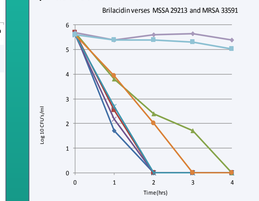
CFU/mL after exposure of E. coli D32 or S. aureus 27660 to brilacidin.

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 978-236-8717 info@cellceutix.com
More information on this and related projects can be obtained at www.cellceutix.com