Brilacidin

First-in-Class Defensin-Mimetic Drug Candidate

Background and Scientific Rationale for Brilacidin as a Potential Novel Coronavirus (COVID-19) Treatment

Updated March 1, 2020

Brilacidin is one of the few drugs targeting COVID-19 that has been tested in clinical trials for other clinical indications, potentially enabling its expedited use against COVID-19

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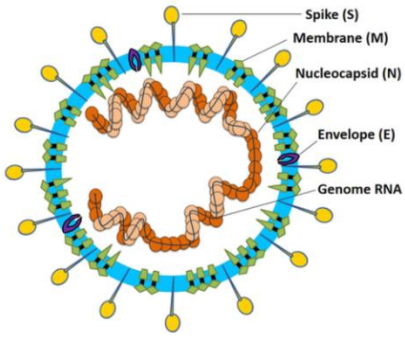
Brilacidin and COVID-19

Brilacidin (PMX-30063) is Innovation Pharmaceuticals' defensin-mimetic drug candidate exhibiting substantial therapeutic potential in human clinical trials.

Defensin-mimetic therapeutics, informed by the attributes of natural defensins (*small antimicrobial peptides, AMPs, expressed widely in the animal kingdom that serve as the first line of defense against foreign invasion of the body*), **comprise an attractive potential therapeutic intervention to combat the coronavirus (COVID-19)** given their innate and multifaceted immunomodulatory properties.

Brilacidin, and other defensin-mimetics similar in structure, have been tested against various clinical isolates of both gram-positive and gram-negative pathogens, with strong activity exhibited across multiple isolates. Enveloped viruses, like the coronavirus, have also been tested, with activity noted. University of Pennsylvania scientists who conducted early Brilacidin research are optimistic about the multi-tiered advantages of Brilacidin to elicit a response, particularly when accompanied by drug optimization, formulation and delivery work, which is supported in the academic literature.

The two Review Articles on coronaviruses, linked below, suggest immunomodulators, like Brilacidin, might be therapeutic options, potentially acting synergistically when combined with other antivirals. The antimicrobial peptide rhesus θ-defensin 1 already has been shown to have an anti-SARS-CoV effect.

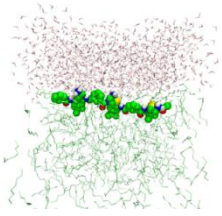


- Geng, Li, et al. "Coronavirus Infections and Immune Responses." *Journal of Medical Virology* (Vol 92, Issue 4): 424-432. doi.org/10.1002/jmv.25685 (January 25, 2020).
- Zumia, A., et al. "Coronaviruses - Drug Discovery and Therapeutic Options." *Nat Rev Drug Discov*. 2016 May;15(5):327-47. doi: 10.1038/nrd.2015.37. Epub 2016 Feb 12.

Exhibiting anti-bacterial, anti-inflammatory and immunomodulatory properties, Brilacidin has shown therapeutic benefit in successfully completed Phase 2 clinical trials in different clinical indications:

- **Acute Bacterial Skin and Skin Structure Infections** (ABSSSI) (FDA QIDP): Phase 2b (NCT02052388), *intravenous delivery*
- **Inflammatory Bowel Disease** (IBD): Phase 2 Proof-of-Concept in Ulcerative Proctitis/Proctosigmoiditis, *enema formulation*; being developed as an *oral tablet* in Ulcerative Colitis, Phase 2 planning underway
- **Oral Mucositis** (OM) (FDA Fast Track): Phase 2 (NCT02324335), *oral rinse delivery*

Supported in the academic literature, potential extension of Brilacidin as a *topical* agent into dermatology indications, such as Atopic Dermatitis and Acne, is planned.



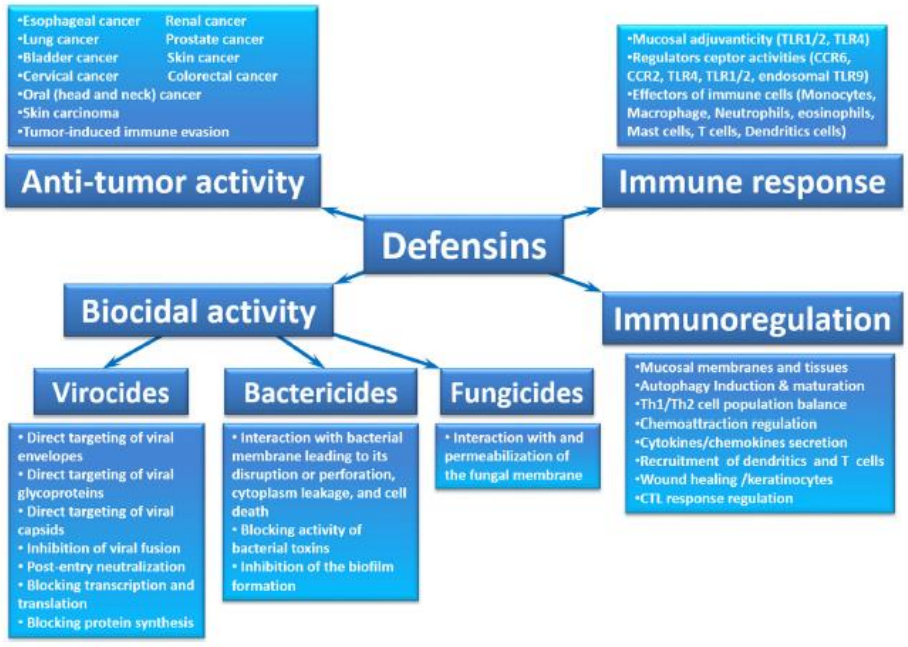
Computationally-modeled after natural Host Defense Proteins/Peptides (HDPs)[1], the "front-line" of defense in the body's innate immune system, Brilacidin is a synthetic, non-peptidic small molecule that kills pathogens swiftly and effectively. Just as importantly, Brilacidin functions in a robust immunomodulatory capacity, lessening inflammation and promoting healing. Based on research originally conducted at the

[1] Brilacidin was modeled on the host defense properties a frog peptide called *magainin* based on research conducted in the late 1980s by Dr. Michael Zasloff. See "New Weapons for the Germ Wars" (pdf) (2002).

University of Pennsylvania—including by two National Academy of Science members (Dr. William F. DeGrado, referred to by *Forbes* in 2011 as the "Antibiotic Artisan" and "Genius Chemist", and Dr. Michael L. Klein), and other scientists—Brilacidin was designed to be *smaller* (1/10th the size), *more stable*, *more potent* (by a 100-fold) and *more selective* (by a 1000-fold) than natural defensins so as to overcome most shortcomings (e.g., degradation, toxicity, malabsorption, high-cost of manufacturing), that have complicated their clinical development.

Defensin/AMP-based-mimetics—again, such as Brilacidin, which was designed *de novo* to optimize drug exposure in terms of pharmacokinetics and efficacy—are increasingly being recognized within Pharma and Academia alike as a "multifaceted" and highly promising class of drug candidates with potential application in treating numerous illnesses and diseases.

Referred to as the "Swiss Army Knife" of the human body, defensins exhibit a number of distinct and highly favorable therapeutic characteristics—*anti-bacterial*, *anti-inflammatory*, *anti-fungal*, *anti-biofilm*, *anti-cancer*, and **antiviral**, across the innate and adaptive immune response. The antiviral properties of natural defensins/AMPs (and their synthetic mimics) are being studied by virologists the world over, with newer understandings elucidating their direct mechanisms of action against non-enveloped and enveloped viruses alike, along with their role in the regulation of inflammation and chemoattraction.



Relevant review articles, with select excerpts, are inked below:

- Park MS, et al. "Towards the Application of Human Defensins at Antivirals." *Biomol Ther* (Seoul). 2018 May 1;26(3):242-254. doi: 10.4062/biomolther.2017.172.

  "Due to the relative nonspecificity of the targets of defensins compared to those of the adaptive arm, antiviral applications of ***defensins are conceptually ideal for defense against different viral infections****." (emphasis added) […]*

  *"****We propose a prophylactic 'defensin vaccine' concept*** *of a planned and controlled overexpression of defensins, which is akin to manually operating the 'safety lock' of natural defensin expression program as needed. Our proposal is in the same conceptual line of Edward Jenner's 'vaccination' (Morgan and Parker, 2007), which took advantage of the inherent human immune system."*

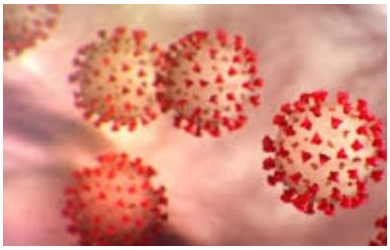
- Ahmed A, et al. "Human Antimicrobial Peptides as Therapeutics for Viral Infections." *Viruses.* 2019 Aug 1;11(8). pii: E704. doi: 10.3390/v11080704.

  "Progress has been made in the last decade to elucidate the mechanisms of action of various AMPs. The primary mechanism of AMP-mediated antiviral activity has been attributed to direct interference with, and destabilization of, viral envelopes. However, AMPs have also demonstrated selective immune modulation. Antiviral activity against both enveloped and non-enveloped viruses has been reported with the latter hinting at the presence of undiscovered activities of AMPs, in addition to the known direct interaction with viral envelopes. […] ***In vulnerable individuals, prophylactic expression of AMPs has the potential to become a preventative strategy against viral infections, especially during emerging pandemics. In addition, the simplicity of AMPs makes the development of synthetic peptide analogues a cost-effective measure to treat established viral infections.*** *[emphasis added] AMPs and their synthetic derivatives are a promising avenue to yield new strategies to control and treat a wide range of viral diseases but their application is still at the preliminary stages. Therefore, further research is warranted to understand AMP antiviral activity both in vivo and in vitro and to determine underlying mechanisms involved in AMP-mediated immune modulation for clinical applications."*

Table 1. Mechanisms of actions of antiviral AMPs.

AMP Family	Target	Proposed Mechanism of Action	References
Defensins	HAdV HIV HSV RSV HPV	Direct interaction with virions; reduction of cell trafficking; direct binding to cell receptor blocking entry (HS); inhibition of protein kinase C signaling; release inhibition of viral components from endosomes; decrease in proinflammaktory cytoine production.	[8,10,13,16,21–28,30–41]
Cathelicidin (LL-37)	HIV DENV RSV HRV VACV HSV ZIKV HCV VEEV	Direct interaction with virions; Increase in type I IFN expression; decrease in proinflammatory cytokine production.	[9,11,37,41,43,44,46–67,70,163]
Transferrin	RSV IAV HPIV HAdV HSV HCV HBV HIV Hantavirus HPV Rotavirus JEV SFV SINV DENV	Direct interaction with virions; inhibition of viral attachment/absorption; delay in viral protein synthesis; Inhibition of cellular trafficking; direct binding to cell receptor blocking entry (HS and DC-SIGN).	[71–118,164]
Eosinophil proteins	RSV HV	Direct interaction with virions	[119–124]
AMPS from Immune cells	HSV HIV	Direct interaction with virions; increase in type I IFN expression	[125–135]
Hepcidin	HBV HCV HIV	Sequester iron from pathogens	[136–141]
Antimicrobial Neuropeptides	HIV	Inhibition of NF-kB and cytokine production	[142–146]

Coronavirus (SARS-CoV-2) (COVID-19)



The coronavirus (COVID-19) outbreak poses a significant life-threatening and economic risk throughout the world. As of March 1, 2020, almost 87,500 cases have been diagnosed across at least 60 countries (now in all continents except Antarctica), including 70 cases in the U.S., resulting in nearly 3,000 reported deaths.

The underlying theory is that Brilacidin, as a defensin-mimetic, might show particular promise as a coronavirus treatment by bolstering the body's natural immune response to help fight the virus[2]—whether administered intravenously[3] as a monotherapy or as an adjuvant therapy—as well as resolve secondary infections in the sickest patients.[4] As previously referenced, defensin-based vaccines have been proposed, though developing Brilacidin as a vaccine would be a longer process (12+ months).

Innovation is exploring collaborations with leading virologists and scientists to further evaluate Brilacidin as a potential novel coronavirus treatment. A Material Transfer Agreement (MTA) has been signed with a Regional Biocontainment Lab. A separate MTA has been submitted to a top University Virology Lab managed by one the world's leading coronavirus experts. Brilacidin drug substance will be sent to assess and inform, at no cost to the Company, the drug's potential antiviral and anti-inflammatory properties in the context of viral infections, including its potential inhibition of SARS-CoV-2, the virus responsible for COVID-19. The Company is making plans potentially to test additional defensin-mimetic compounds (synthetic analogs similar to Brilacidin) that might be tailored to exhibit enhanced antiviral activity.

U.S. Department of Health & Human Services
Public Health Emergency

Medical Countermeasures.gov

The Company has submitted a preliminary summary of Brilacidin's potential as a novel coronavirus treatment to the Biomedical Advanced Research and Development Authority (BARDA), dedicated to rapidly identifying and funding medical countermeasures to COVID-19. Should lab tests support Brilacidin's antiviral activity against SARS-CoV-2 (COVID-19), and potentially other RNA viruses, the Company may look to expedite clinical development of Brilacidin as an antiviral therapy, including targeting COVID-19, via pharmaceutical partnerships, academic collaborations and government grants.

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[2] Brilacidin, shown in pre-clinical tests to inhibit PDE4/PDE3, might provide added benefit as a result of this particular mechanism by 1) disrupting viral replication; and 2) enhancing the protective role of natural surfactants in the lung, helping resolve respiratory problems common to the coronavirus. Defensins play a key role in pulmonary and mucosal host defense. Expression levels of defensins in the body have also been studied as promising pathogen-specific biomarkers.

[3] Brilacidin completed a Phase 2b FDA trial where, delivered as a single dose, it compared favorably to a 7-day dosing regimen of daptomycin (Cubicin™). See mechanistic studies of Brilacidin: Mensa, B., et al. (2014).

[4] According to available epidemiological and clinical data, patients most severely affected by COVID-19—up to 20% of cases, usually the elderly and those with underlying conditions—can require hospitalization and intensive care. These patients exhibit higher levels of pro-inflammatory cytokines, which Brilacidin inhibits (e.g., *anti-TNF-α*, *IL-1β*, *IL-6*, *IL-8, MIP2-α*, *MCP-1, MMP-9*). Bacterial infections can co-present in these patients. Brilacidin may help fight such infections given its strong anti-infective activity against multiple strains of pathogenic bacteria (see: "Brilacidin as a Late-Stage Antibiotic Drug Candidate"), including methicillin-resistant Staphylococcus aureus (MRSA). The duration of antibiotic treatment, in one study of 99 cases in Wuhan China, was between 3 and 17 days (a median of 5 days). Single-dose Brilacidin might help reduce the time to possible disease resolution toward preventing deaths. Highly transmissible (asymptomatic spreading), COVID-19 has a ~2 to 3% mortality rate.

What you need to know about coronavirus disease 2019 (COVID-19)

What is coronavirus disease 2019 (COVID-19)?

Coronavirus disease 2019 (COVID-19) is a respiratory illness that can spread from person to person. The virus that causes COVID-19 is a novel coronavirus that was first identified during an investigation into an outbreak in Wuhan, China.

Can people in the U.S. get COVID-19?

COVID-19 is spreading from person to person in China, and limited spread among close contacts has been detected in some countries outside China, including the United States. At this time, however, this virus is NOT currently spreading in communities in the United States. Right now, the greatest risk of infection is for people in China or people who have traveled to China. Risk of infection is dependent on exposure. Close contacts of people who are infected are at greater risk of exposure, for example health care workers and close contacts of people who are infected with the virus that causes COVID-19. CDC continues to closely monitor the situation.

Have there been cases of COVID-19 in the U.S.?

Yes. The first case of COVID-19 in the United States was reported on January 21, 2020. The current count of cases of COVID-19 in the United States is available on CDC's webpage at https://www.cdc.gov/coronavirus/2019-ncov/cases-in-us.html.

How does COVID-19 spread?

The virus that causes COVID-19 probably emerged from an animal source, but now it seems to be spreading from person to person. It's important to note that person-to-person spread can happen on a continuum. Some diseases are highly contagious (like measles), while other diseases are less so. At this time, it's unclear how easily or sustainably the virus that causes COVID-19 is spreading between people. Learn what is known about the spread of newly emerged coronaviruses at https://www.cdc.gov/coronavirus/2019-ncov/about/transmission.html.

What are the symptoms of COVID-19?

Patients with COVID-19 have had mild to severe respiratory illness with symptoms of

- fever
- cough
- shortness of breath

What are severe complications from this virus?

Many patients have pneumonia in both lungs.

How can I help protect myself?

The best way to prevent infection is to avoid being exposed to the virus that causes COVID-19.

There are simple everyday preventive actions to help prevent the spread of respiratory viruses. These include

- Avoid close contact with people who are sick.
- Avoid touching your eyes, nose, and mouth with unwashed hands.
- Wash your hands often with soap and water for at least 20 seconds. Use an alcohol-based hand sanitizer that contains at least 60% alcohol if soap and water are not available.

If you are sick, to keep from spreading respiratory illness to others, you should

- Stay home when you are sick.
- Cover your cough or sneeze with a tissue, then throw the tissue in the trash.
- Clean and disinfect frequently touched objects and surfaces.

What should I do if I recently traveled to China and got sick?

If you were in China within the past 14 days and feel sick with fever, cough, or difficulty breathing, you should seek medical care. Call the office of your health care provider before you go, and tell them about your travel and your symptoms. They will give you instructions on how to get care without exposing other people to your illness. While sick, avoid contact with people, don't go out and delay any travel to reduce the possibility of spreading illness to others.

Is there a vaccine?

There is currently no vaccine to protect against COVID-19. The best way to prevent infection is to avoid being exposed to the virus that causes COVID-19.

Is there a treatment?

There is no specific antiviral treatment for COVID-19. People with COVID-19 can seek medical care to help relieve symptoms.

For more information: **www.cdc.gov/COVID19**

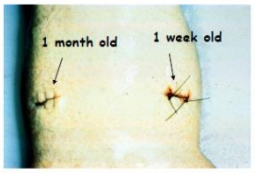
Source

Antimicrobial Peptides in Health and Disease

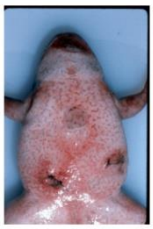
Michael Zasloff, MD., Ph.D.

Microbial Ecology in States of Health and Disease
Forum on Microbial Threats
March 18-19, 2013
Washington, DC

Discovery of Magainin Peptides



Usual healing



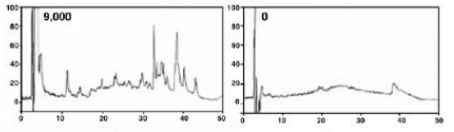
The rare case

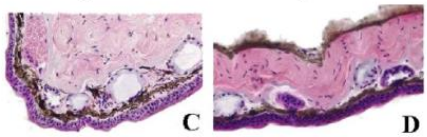
Environmental microbes needed for regeneration of antimicrobial peptide skin arsenal

Frogs returned to "normal" aquarium

Frogs returned to sterile water + antibiotics

2 weeks later





Fundamental Design Principle: Amphipathic Distribution of Cationic and Hydrophobic Amino Acids

Human Neutrophil Defensin 3

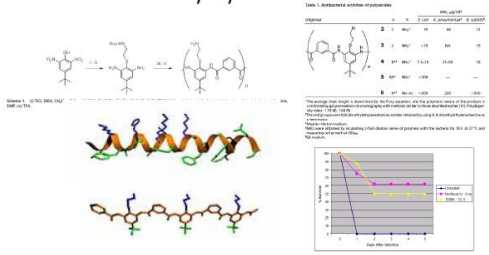
Indolicidin

Magainin 2

Protegrin

Antimicrobial peptide mimetics

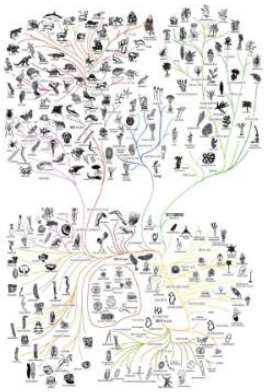
Polyarylamides



Adopts amphipathic secondary structures

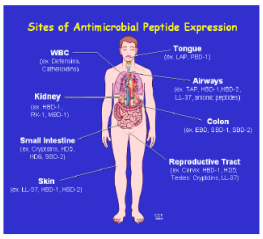
S. aureus, in vivo

DeGrado and Klein, UPenn

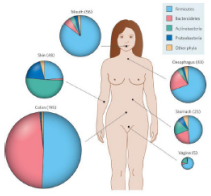


Over 1000 different antimicrobial peptides have been discovered in animals and plants

Antimicrobial peptides are produced in the body wherever we find microbes

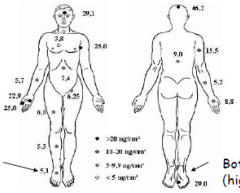


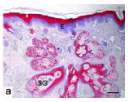
Different types and numbers of microbes in different parts of the body



Concentrations of specific AMPs on human skin match the degree of protection needed:

Psoriasin, as an example





Top of foot (low)

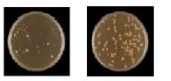
Bottom of foot (high)

Psoriasin surface concentrations

Modified from (Glaser et al, Nat Immunology, 2005)

Most soaps wash off the powerful AMPs on our skin.

After using soap, remaining E. coli can actually grow more rapidly than before!!



Unwashed

Soap-washed

10 minutes

Source (pdf); See also: Zasloff, M. "Antimicrobial Peptides of Multicellular Organisms: My Perspective." *Adv Exp Med Biol*. 2019;1117:3-6. doi: 10.1007/978-981-13-3588-4_1.

Host defense (antimicrobial) peptides

10

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10.1 Overview of host defense peptides

The increasing threat of antibiotic resistance and emergence of multidrug-resistant bacteria in hospital- and community-acquired infections is a growing medical concern. In 2014, the World Health Organization released a global report on antimicrobial resistance emphasizing the increasing threat posed by resistant bacterial, parasitic, viral, and fungal pathogens and suggested that a postantibiotic era may be on the horizon [1]. Subsequently, in 2016 the United Nations recognized the threat posed by antimicrobial resistance to human health, development, and global stability, and committed to foster innovative ways to address this global threat [2]. One promising antiinfective approach is the use of antimicrobial peptides (AMPs). These are short polypeptides found in all species of complex life including plants, insects, crustaceans, and animals (including humans), and are integral components of their innate immune systems [3,4]. Originally appreciated for their direct antimicrobial activity against planktonic bacteria [5], natural AMPs have also been shown to have potent immunomodulatory functions both in vitro and in vivo [5]. Therefore, we prefer to use the term host defense peptide (HDP) to describe these molecules to better reflect the broad range of biological activities that they mediate.

Individual HDPs can exhibit a wide range of activities that are uniquely determined, but often overlapping within a single molecule. These activities encompass various functions including direct antimicrobial activity towards bacteria, viruses, and fungi, antibiofilm activity as well as a variety of immunomodulatory functions. Here we summarize the different types of activities that have been observed for natural and synthetic HDPs, and highlight current and future applications of these multifaceted molecules with a particular emphasis on their potential use as novel antiinfective agents.

Source (pdf)

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Multifaceted immune functions of human defensins and underlying mechanisms

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Abstract

Defensins have been long recognized as natural antimicrobial peptides, but they also possess diverse and versatile immune functions. Defensins can both induce inflammation and suppress inflammatory responses by acting on specific cells through distinct mechanisms. Defensins can also modulate the immune response by forming a complex with cellular molecules including proteins, nucleic acids, and carbohydrates. The mechanisms of defensin-mediated immune modulation appear to be cell-type and context specific. Because the levels of human defensins are often altered in response to infection or disease states, suggesting their clinical relevance, this review summarizes the complex immune functions of human defensins and their underlying mechanisms of action, which have implications for the development of new therapeutics.

Keywords

Defensins; Immune functions; Pathways

1. Introduction

Defensins are antimicrobial peptides known to protect the host through their direct or indirect activities on microbes [1–3], although recent studies have demonstrated their ability to promote viral infectivity [4,5], indicating a complex role of defensins in host defense in a microbe, defensin, cell-type specific manner. As major players at the front line of defense, there has been much discussion of the immunological activities of defensins and their role as alarmins in host defense [1,6–8]. Here, we focus on the immune functions of human defensins that are beneficial or detrimental to the host. We highlight advances in our understanding of the molecular mechanisms of immune modulatory activities of human defensins.

Source (pdf)

Cite this: *Polym. Chem.*, 2018, **9**, 2407

Biomimetic antimicrobial polymers: recent advances in molecular design

Cansu Ergene,[a] Kazuma Yasuhara[b] and Edmund F. Palermo *[a]

The increasing prevalence of antibiotic-resistant bacterial infections, coupled with the decline in the number of new antibiotic drug approvals, has created a therapeutic gap that portends an emergent public health crisis. Since the 1980s, host defense peptides (HDPs) have been recognized as antibacterial compounds that do not induce resistance, but are hampered by their high cost and lack of synthetic scalability. Starting in the early 2000s, synthetic (co)polymers have been designed to mimic the salient physiochemical features of HDPs. These polymers have shown broad-spectrum antimicrobial activity, rapid bactericidal kinetics, and a very low propensity to induce resistance. Systematic optimization of the (co)polymer composition, chain length, hydrophobicity, and cationic charge has generated select examples that are also highly biocompatible (non-hemolytic and non-cytotoxic *in vitro*). These polymers are derived from inexpensive feedstocks and are produced using cost-effective, scalable processes. Accordingly, such polymers may be viewed as early stage pre-clinical candidates for potential use in pharmaceutical or therapeutic applications. In this review, we focus on the key macromolecular design principles that have been gleaned from more than a decade of structure–activity relationship (SAR) studies, as well as some key mechanistic investigations, across this multidisciplinary field. A fundamental understanding of these functional (co)polymers has arisen from a convergence of ideas in polymer chemistry, microbiology, and biophysics. In this context, we emphasize the recent advances from the past few years and emerging opportunities surrounding the rapidly growing field of HDP-mimetic antimicrobial polymers.

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rsc.li/polymers

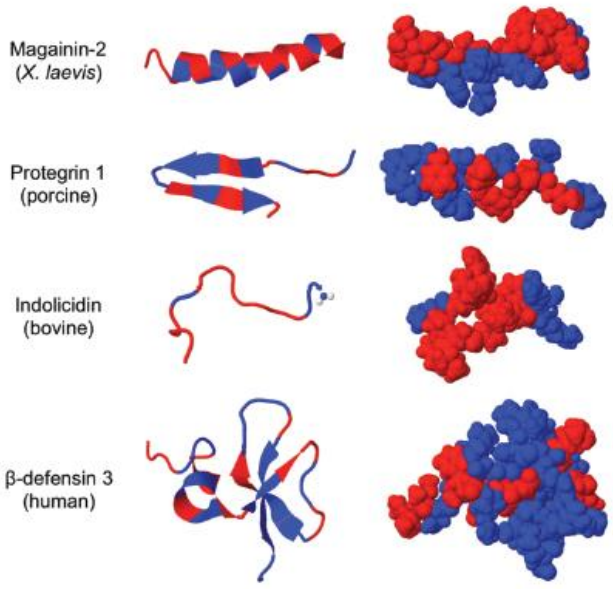
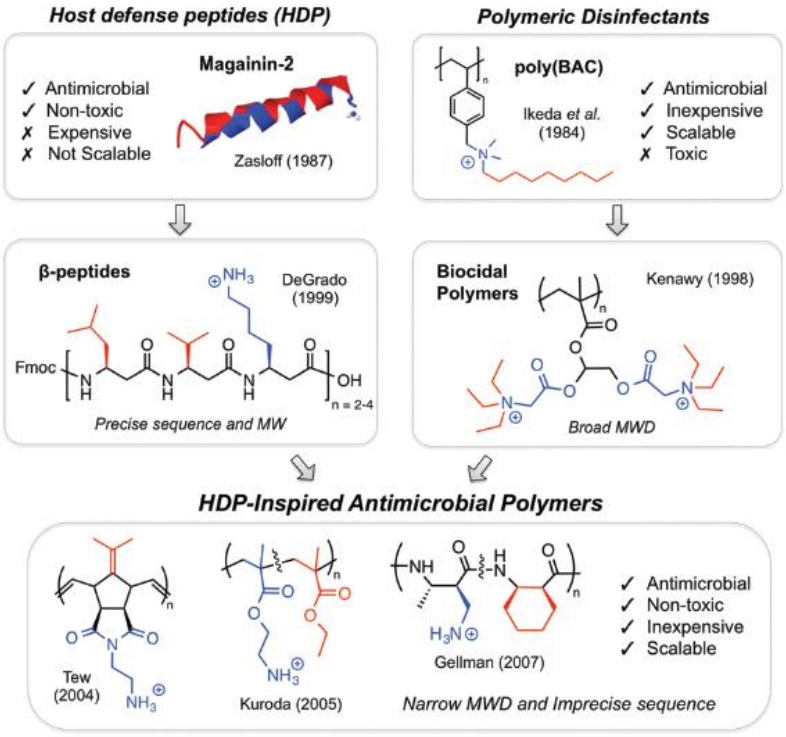


Fig. 1 Molecular models of four HDPs. The hydrophobic residues are red and the hydrophilic residues are blue. There is a lack of conserved secondary structure but hydrophobic residues appear to cluster into distinct domains. The images were generated using the RCSB Protein Databank (http://www.rcsb.org/pdb/home/home.do).



Source (pdf)

Current Topics in Medicinal Chemistry, 2017, *17*, 576-589

REVIEW ARTICLE

Mimics of Host Defense Proteins; Strategies for Translation to Therapeutic Applications

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ARTICLE HISTORY

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Abstract: New infection treatments are urgently needed to combat the rising threat of multi-drug resistant bacteria. Despite early clinical set-backs attention has re-focused on host defense proteins (HDPs), as potential sources for new and effective antimicrobial treatments. HDPs appear to act at multiple targets and their repertoire includes disruptive membrane and intracellular activities against numerous types of pathogens as well as immune modulatory functions in the host. Importantly, these novel activities are associated with a low potential for emergence of resistance and little cross-resistance with other antimicrobial agents. Based on these properties, HDPs appear to be ideal candidates for new antibiotics; however, their development has been plagued by the many therapeutic limitations associated with natural peptidic agents. This review focuses on HDP mimetic approaches aimed to improve metabolic stability, pharmacokinetics, safety and manufacturing processes. Early efforts with β-peptide or peptoid analogs focused on recreating stable facially amphiphilic structures but demonstrated that antimicrobial activity was modulated by more, complex structural properties. Several approaches have used lipidation to increase the hydrophobicity and membrane activity. One lead compound, LTX-109, has entered clinical study as a topical agent to treat impetigo and nasal decolonization. In a more significant departure from the amino acid like peptidomimetics, considerable effort has been directed at developing amphiphilic compounds that recapitulate the structural and biological properties of HDPs on small abiotic scaffolds. The lead compound from this approach, brilacidin, has completed two phase 2 studies as an intravenous agent for skin infections.

Keywords: Antibiotic, Antimicrobial peptides, Antiseptic, Host defense proteins, *In vivo*, LPS, LTA, Membrane, Mimetics, Mimics, Pharmacokinetics, Resistance, Therapeutic, TNF.

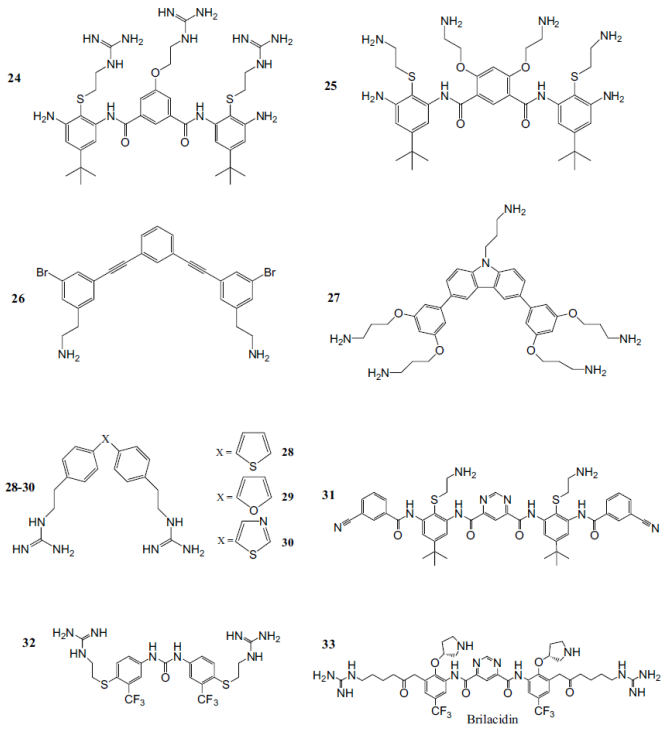

Fig. (6). Structures of smHDPs.

Source (pdf)

JOURNAL OF VIROLOGY, Nov. 2009, p. 11385–11390 Vol. 83, No. 21
0022-538X/09/$12.00 doi:10.1128/JVI.01363-09

Rhesus Theta-Defensin Prevents Death in a Mouse Model of Severe Acute Respiratory Syndrome Coronavirus Pulmonary Disease▽

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Departments of Pediatrics,[1] Pathology,[2] Internal Medicine,[3] and Microbiology,[4] Carver College of Medicine, University of Iowa, Iowa City, Iowa 52242, and Department of Pathology and Laboratory Medicine, University of California Irvine, Irvine, California 92697[5]

Received 2 July 2009/Accepted 13 August 2009

We evaluated the efficacy of rhesus theta-defensin 1 (RTD-1), a novel cyclic antimicrobial peptide, as a prophylactic antiviral in a mouse model of severe acute respiratory syndrome (SARS) coronavirus (CoV) lung disease. BALB/c mice exposed to a mouse-adapted strain of SARS-CoV demonstrated 100% survival and modest reductions in lung pathology without reductions in virus titer when treated with two intranasal doses of RTD-1, while mortality in untreated mice was ~75%. RTD-1-treated, SARS-CoV-infected mice displayed altered lung tissue cytokine responses 2 and 4 days postinfection compared to those of untreated animals, suggesting that one possible mechanism of action for RTD-1 is immunomodulatory.

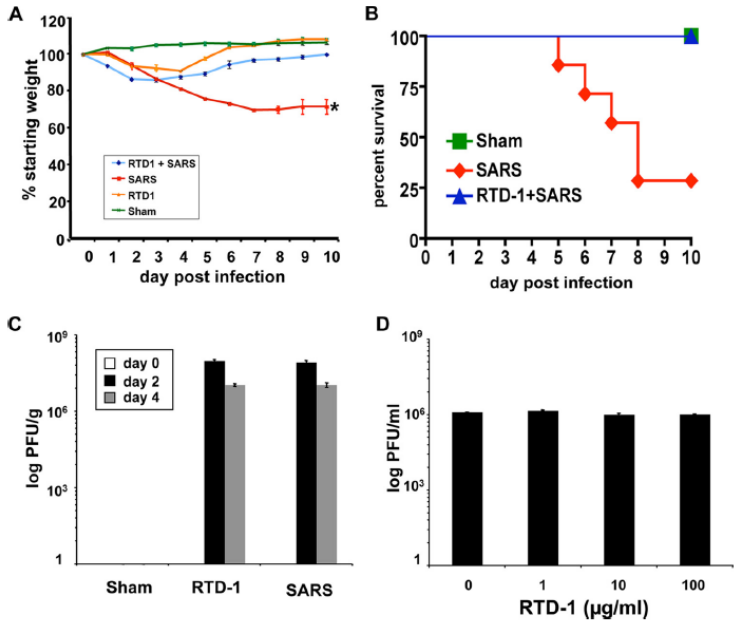

FIG. 1. Treatment with RTD-1 protects mice against lethality of SARS-CoV infection. (A and B) BALB/c mice 6 to 8 weeks old were treated with sham control (40 μl phosphate-buffered saline, no virus), RTD-1 alone (125 μg [~5 mg/kg] intranasal RTD-1 15 min prior to infection, followed by an identical dose 18 h later), SARS-CoV alone (3×10^5 PFU MA15 intranasally in 40 μl phosphate-buffered saline), or RTD-1 followed with SARS-CoV infection. Mice were monitored daily for weight loss (A) and survival (B) ($n = 6$ or 7/group). SARS-CoV-infected mice without RTD-1 treatment had a 30% survival rate and 25% decrease in weight in those that survived (*, $P \leq 0.05$ by Student's t test for SARS alone versus all other groups). Data presented in panels A and B are representative of two independent experiments. (C) Lung tissue was harvested from mice, and viral titer levels were determined. (D) RTD-1 has no direct antiviral effect on SARS-CoV. Viral titers were determined using Vero cells and samples of 1×10^5 PFU SARS-CoV (Urbani) that were preincubated for 30 min at 37°C with RTD-1 in serum-free phosphate-buffered saline at the indicated concentrations prior to plaque assay in Vero cells. Results in panels A, C, and D are presented as means ± standard errors (A and B, $n = 6$ or 7; C and D, $n = 3$). All experiments were performed under biosafety level 3 containment. This study was approved by the University of Iowa Animal Care and Use Committee.

Source (pdf)

Evaluating the Value of Defensins for Diagnosing Secondary Bacterial Infections in Influenza-Infected Patients

Siyu Zhou[1], Xianwen Ren[2], Jian Yang[1]* and Qi Jin[1]**

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Acute respiratory infections by influenza viruses are commonly causes of severe pneumonia, which can further deteriorate if secondary bacterial infections occur. Although the viral and bacterial agents are quite diverse, defensins, a set of antimicrobial peptides expressed by the host, may provide promising biomarkers that would greatly improve the diagnosis and treatment. We examined the correlations between the gene expression levels of defensins and the viral and bacterial loads in the blood on a longitudinal, precision-medical study of a severe pneumonia patient infected by influenza A H7N9 virus. We found that DEFA5 is positively correlated to the blood load of influenza A H7N9 virus ($r = 0.735$, $p < 0.05$, Spearman correlation). DEFB116 and DEFB127 are positively and DEFB108B and DEFB114 are negatively correlated to the bacterial load. Then the diagnostic potential of defensins to discriminate bacterial and viral infections was evaluated on an independent dataset with 61 bacterial pneumonia patients and 39 viral pneumonia patients infected by influenza A viruses and reached 93% accuracy. Expression levels of defensins in the blood may be of important diagnostic values in clinic to indicate viral and bacterial infections.

Keywords: viral infection, bacterial infection, diagnosis, defensin, gene expression

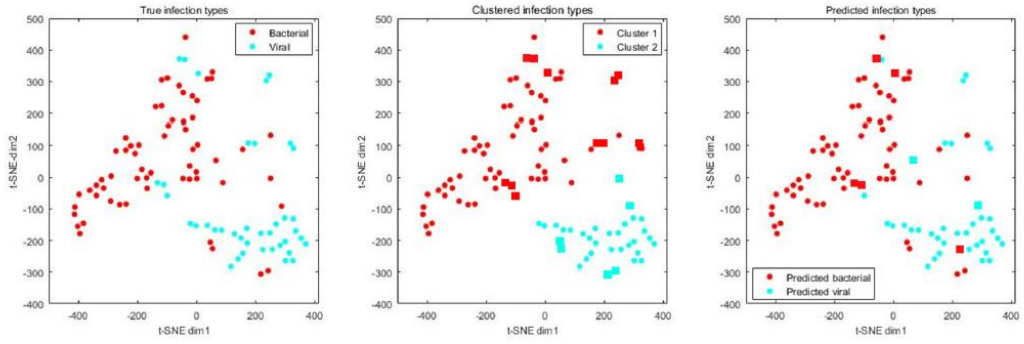

FIGURE 2 | True, clustered and predicted infection types of 61 bacterial and 39 viral pneumonia patients. Expression levels of defensins and associated genes were extracted from the whole dataset and then subjected to t-SNE analysis for visualization. Circles mean correctly clustered/classified samples while rectangles mean incorrectly clustered/classified samples.

Source

BIOMOLECULES & THERAPEUTICS

Review
Biomol Ther 26(3), 242-254 (2018)

Towards the Application of Human Defensins as Antivirals

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Abstract

Defensins are antimicrobial peptides that participate in the innate immunity of hosts. Humans constitutively and/or inducibly express α- and β-defensins, which are known for their antiviral and antibacterial activities. This review describes the application of human defensins. We discuss the extant experimental results, limited though they are, to consider the potential applicability of human defensins as antiviral agents. Given their antiviral effects, we propose that basic research be conducted on human defensins that focuses on RNA viruses, such as human immunodeficiency virus (HIV), influenza A virus (IAV), respiratory syncytial virus (RSV), and dengue virus (DENV), which are considered serious human pathogens but have posed huge challenges for vaccine development for different reasons. Concerning the prophylactic and therapeutic applications of defensins, we then discuss the applicability of human defensins as antivirals that has been demonstrated in reports using animal models. Finally, we discuss the potential adjuvant-like activity of human defensins and propose an exploration of the 'defensin vaccine' concept to prime the body with a controlled supply of human defensins. In sum, we suggest a conceptual framework to achieve the practical application of human defensins to combat viral infections.

Key Words: Adjuvant, Antiviral, Defensin, Prophylactic, Therapeutic, Virus

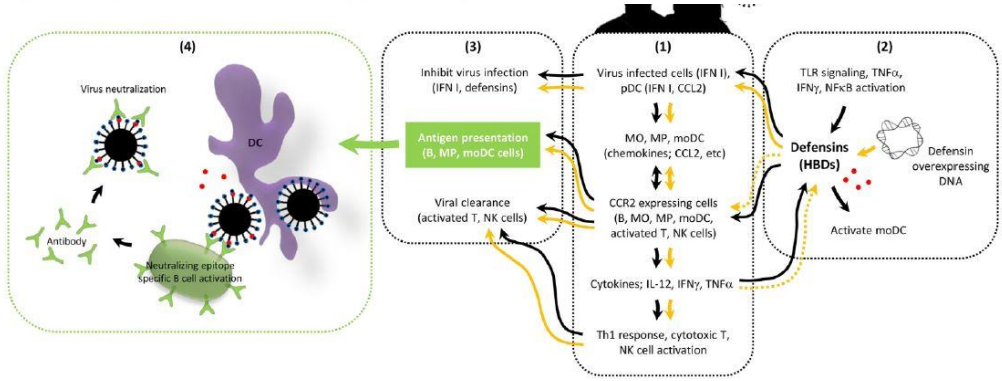

Fig. 2. Postulated mechanism of antiviral defense by a prophylactic defensin overexpression 'vaccine.' Local responses surrounding the infected cells after viral entry into the human body are depicted. (1) Virus infection-associated recruitment of innate and adaptive immune cells are depicted (Watford *et al*., 2003; Megjugorac *et al*., 2004; Hokeness *et al*., 2005; Crane *et al*., 2009; Rohrl *et al*., 2010a; Gerlier and Lyles, 2011; Uyangaa *et al*., 2015). (2) Defensin expression (for induced and systemically overexpressed defensins) is depicted (Albanesi *et al*., 2007; Edfeldt *et al*., 2010; Kawai and Akira, 2011); due to the potential cytotoxicity of excess amount of HADs, only HBDs are considered for an interventional application. (3) Viral clearance is depicted. (4) Potential role of defensins in exposing the neutralizing epitope of a virus and the potential rapid T-cell-independent neutralizing epitope-specific naive B cell activation against a virus captured by recruited B cells and dendritic cells (DCs) are postulated (Vos *et al*., 2000; Swanson *et al*., 2010; Pone *et al*., 2012). T-cell-dependent processes can occur similarly in the presence of T cells in the draining lymph nodes (Wykes *et al*., 1998; Gonzalez *et al*., 2010). Black arrows indicate processes affected by constitutively expressed or physiologically induced defensins. Orange arrows indicate the potential amplification of the processes by the overexpressed defensins. Dashed orange arrows indicate the processes not likely to be influenced by the overexpressed defensins due to the systemic nature of their overexpression. However, the concentration of locally induced defensins due to viruses and cytokines might be higher than the systemic concentration of the overexpressed defensins, and there may be a locally enhanced induction loop. Viral infection results in type I interferon (IFN I) production by infected cells and virus-stimulated plasmacytoid dendritic cells (pDCs) (1), which also produce CCL2. Viral infection also induces defensin expression (2). Defensins (2) exaggerate viral RNA-mediated IFN I induction (1). Defensins (2) can bind to CCR2 and act as chemoattractants to CCR2-expressing cells (1). CCR2-expressing monocytes (MO), macrophages (MP) and monocyte-derived dendritic cells (moDCs) respond to IFN I, CCL2, and defensins and produce further CCL2 and further recruit CCR2-expressing B, MO, MP, moDC, activated T, and natural killer (NK) cells (1). These cells produce cytokines, such as IL-12, gamma interferon (IFNγ) and tumor necrosis factor-alpha (TNFα) (1). IL-12 and IFNγ promote Th1 responses and activate cytotoxic T and NK cells (1). IFNγ and TNFα induce defensins (2). During this cycle, the innate arm of defense (IFN I, defensins, and NK cells) inhibits virus replication and removes the virus-infected cells (3). Recruited antigen-presenting cells (B, MP and moDCs) initiate the adaptive arm of defense (3). On-site T cell-independent viral antigen-specific B cell activation and antibody secretion could occur against the virus captured by recruited B cells and DCs (4). Defensin-mediated exposure of the neutralizing epitope of the virus would further enhance neutralizing epitope-specific antibody responses and viral clearance (4). Overexpressed defensins would increase systemic levels of defensins and enhance all of the processes at the location of viral infection to clear the virus, providing a memory response-like effect. Objects in the cartoon are not to scale.

Source (pdf)

Current Protein and Peptide Science, 2005, *6*, 53-60

Defensins - Non-antibiotic Use for Vaccine Development

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Abstract: Vaccines should elicit protective and long lasting immune memory, which depends on well choreographed responses between innate and acquired immunity. Defensins are small host defense peptides of innate immunity hitherto reported to have antimicrobial activity, which also orchestrate chemotaxis and activation of effector immune cells, including immature dendritic cells. This review analyzes the biological meaning of the immunomodulatory and immunoenhancing features of defensins and their use for the development of novel vaccines to combat cancer and clinically relevant diseases.

Keywords: Antimicrobial peptides, dendritic cells, vaccine carrier.

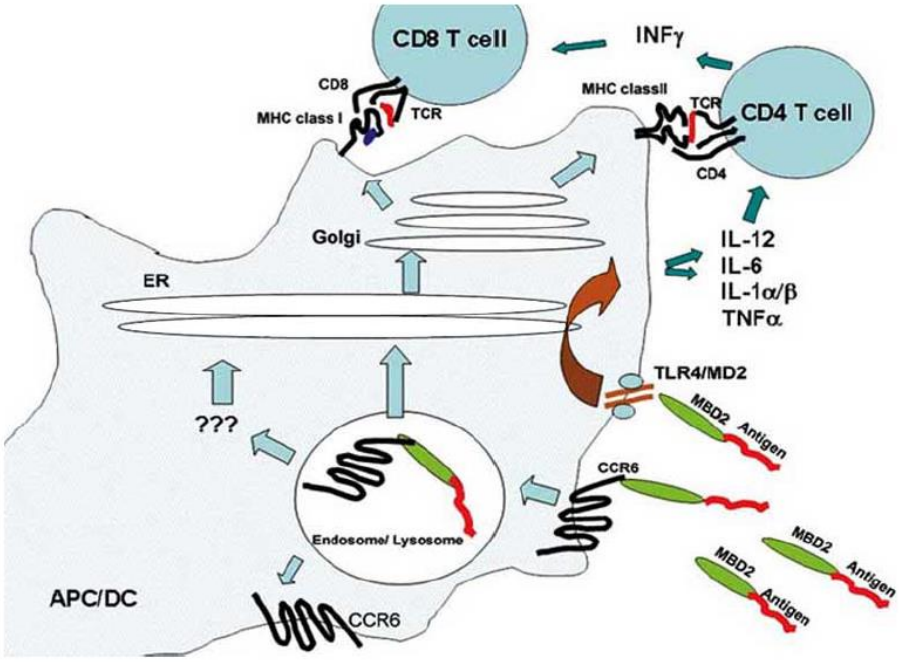


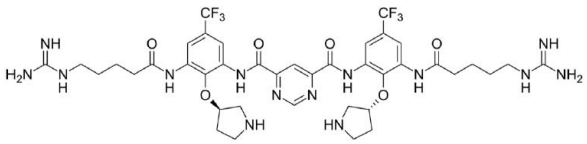
Fig. (1). Defensins as carrier for vaccines to target APCs. Murine β-defensin 2 –fused antigens target to APCs via the CCR6 chemokine receptor CCR6, which is internalized to deliver the complex to early/late endosomal compartments. The internalized defensin-antigen is processed and presented to both MHC class II and MHC class I to elicit CD4 and CD8 T cell responses. At the same time, murine β-defensins 2 fused antigens induce maturation of iDCs and the the production of Th1 polarizing cytokines.

Conclusion: "Taken together, these features of the defensins and other antimicrobial peptides have to be considered when they are utilized as adjuvant and vaccine carriers for non-immunogenic or weakly immunogenic antigens. ***Use of different defensins may enable induction of controlled and polarized immune responses individually tailored for the specific disease at will."*** *[emphasis added]*

Source (pdf)

Host Defense Protein (HDP) Mimics

Chemical Properties and Design of Brilacidin—a Multi-Indication Mid-to-Late Stage HDP-Mimic Drug Candidate



Small, non-peptidic, fully-synthetic mimics of HDPs developed as a systemic or topical agents.
Biocomputational aspects of Brilacidin's development have resulted in the drug candidate exhibiting tailored exposure and efficacy across multiple clinical indications.

Formula $C_{40}H_{50}F_6N_{14}O_6$

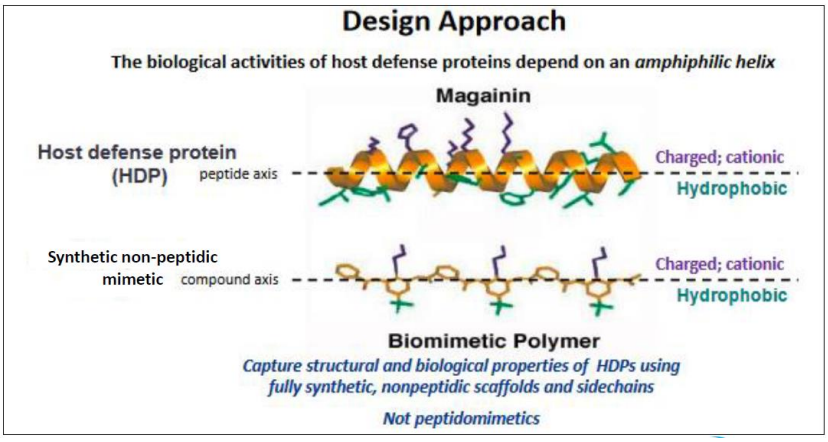
Molar Mass 936.9 g/mol

- **HDPs are Small Antimicrobial Peptides**

-Expressed widely in the animal kingdom
-Produced in skin, mucosal surfaces, neutrophils
-Target microbial membrane

- **First Line of Defense Against Foreign Invasion**

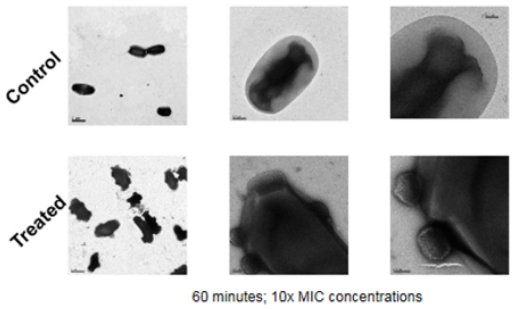
-Part of innate immunity
-Maintenance of epithelial barrier function
-Regulate microbiota
-Immunomodulatory – innate and adaptive immunity
-Anti-infective properties
-Anti-inflammatory properties



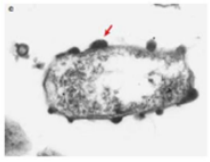
Brilacidin—Mechanism of Action (Anti-Microbial)

Disrupts Microbial Membranes… Leading to Rapid Bacterial Cell Death

Brilacidin functions as an anti-microbial, piercing the cell walls of bacteria (bactericidal)



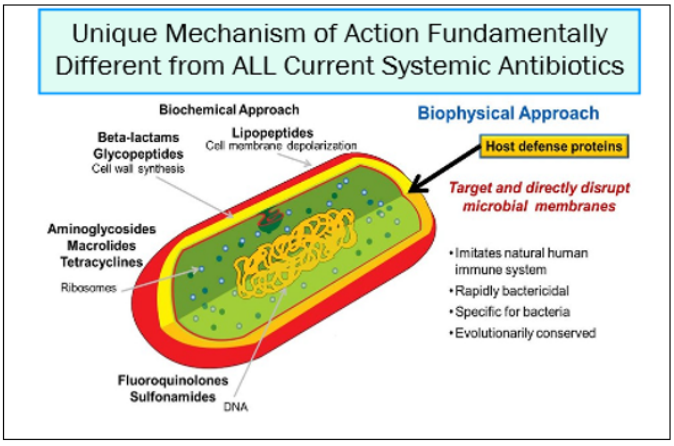
TEM of *P. aeruginosa* on SMAP29 (3 hrs)



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.

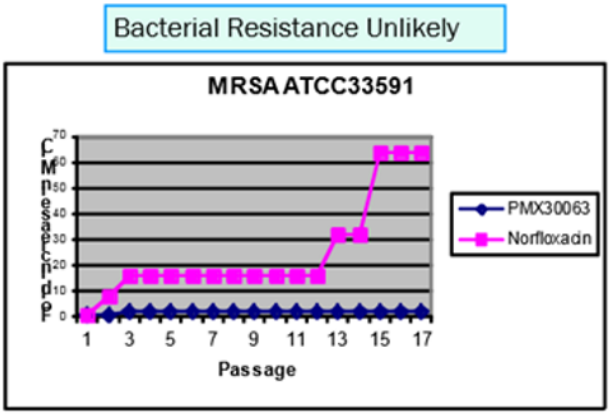
Similar morphological response reported for SMAP29 and P. aeruginosa.



Brilacidin received FDA QIDP designation for ABSSSI (Fast Track, Priority Review); additional 5 years of market exclusivity in the U.S.

Brilacidin—Mechanism of Action (Anti-Microbial)

Rapid Killing Ability Makes Antibiotic Resistance Less Likely



Serial passage resistance studies are used to demonstrate the potential for bacterial resistance to develop to antibiotics. The graph shows brilacidin compared to a conventional antibiotic, norfloxacin for the development of resistance against *MRSA*. ***With brilacidin, no bacterial resistance was seen in up to 40 serial passages.***

Membrane Activity

supported by:

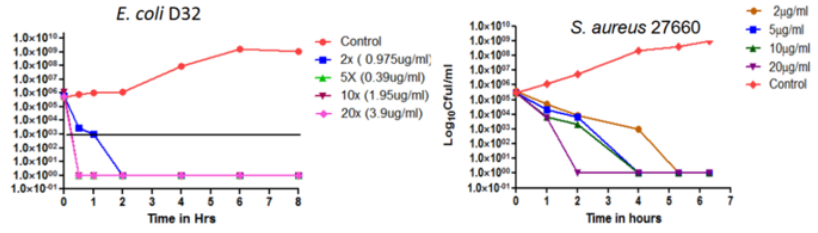
- *Coarse grain molecular dynamic simulations*
- *Vesicle leak assays*
- *Membrane permeabilization and potentiation assays*
- *Transcriptional profiling, proteomics and deep sequencing*
- *Transmission electron microscopy*

Brilacidin—Broad Spectrum Anti-Microbial Coverage

In Vitro Anti-Microbial Activity: Gram+ Activity, Gram- Coverage; Low Cytoxicity 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.

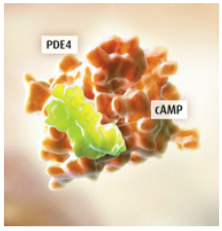
See: http://www.ipharminc.com/new-blog/2018/3/7/brilacidin-as-a-novel-late-stage-antibiotic-drug-candidate

Key Attributes:

- Bactericidal not bacteriostatic
- Intermediate half-life (drug clears the body sooner)
- Active against stationary-phase bacteria common to biofilms and persistent infections
- Robust sub-MIC activity
- Single-dose translates into direct hospital cost savings (fewer inpatient days)
- Single-dose improves patient adherence treatment (fewer readmissions)
  - According to a March 2016 study, just 57% of patients with *S. aureus* infections took their prescribed doses upon hospital discharge, resulting in nearly half of them getting a new infection or needing additional treatment for an existing skin infection.

Brilacidin—Mechanism of Action (Immuno-Inflammatory)

Inhibits PDE4… Lessening Inflammation, Promoting Healing



Brilacidin is a novel immuno-inflammatory/anti-microbial drug candidate that acts through inhibition of PDE4

- Functions through the cyclic AMP/cyclic GMP pathways
- Suppresses pro-inflammatory mediators and increases anti-inflammatory mediators

Brilacidin may also act in a compensatory way in offsetting defensin-related mucosal deficiencies, e.g., in IBD

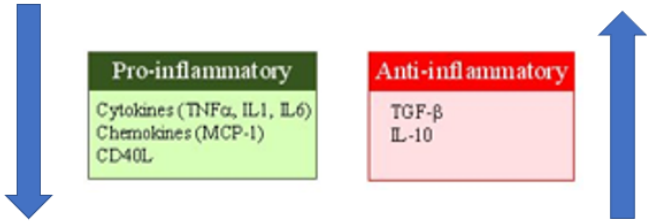
Effectiveness as an antibacterial (systemic) demonstrated in a successful Ph2b **ABSSSI clinical trial

(**ABSSSI - Acute Bacterial Skin and Skin Structure Infection)

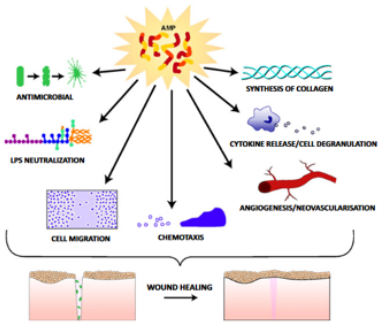
Effectiveness in Oral Mucositis (topical), a large area of unmet need, demonstrated in a successful Phase 2 trial

Planned extension (topical) into Dermatological Disease (Atopic Dermatitis, Acne, H. Suppurativa)

Lessens Inflammation

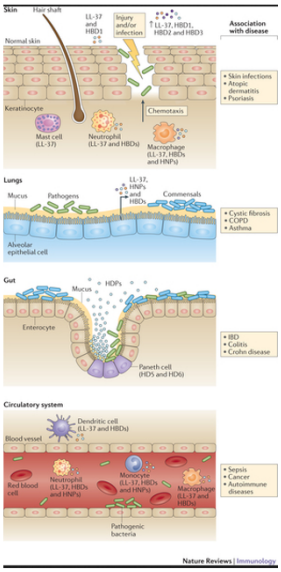


Promotes Healing



Brilacidin—Low Systemic Absorption

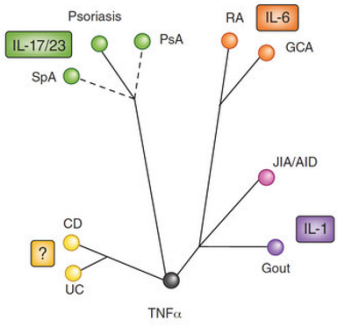
Potential to Treat Multiple Inflammatory Conditions as a Topical Medicine



"Understanding and controlling inflammation has become a central goal of modern medical investigation."

Dr. Jerome Groopman

Brilacidin's low level of systemic exposure as revealed in Phase 2 trials in UP/UPS and OM—measurements of concentrations of Brilacidin in plasma showed all levels, across all time points, to be on the lower limit of quantification, i.e., <200 ng/mL—suggests that multiple chronic inflammatory conditions might be treated locally and efficaciously with the drug as a topical medicine.



CID	TNF	IL-6R	IL-1	IL-12/23	IL-17A
Rheumatoid arthritis					
Giant cell arthritis					
JIA/AID					
Gout					
Crohn's disease					
Ulcerative colitis					
Psoriasis					
Psoriatic arthritis					
Ankylosing spondylitis					
Multiple sclerosis					
Drugs	Adalimumab Certolizumab Etanercept Golimumab Infliximab	Tocilizumab Sarilumab*	Anakinra Canakinumab Rilonacept	Ustekinumab Briakinumab*	Brodalumab* Ixekizumab* Secucinumab*

Sources: "How Cytokine Networks Fuel Inflammation: Toward a Cytokine-Based Disease Taxonomy" (Nature Medicine 19, 833-824 2013); "The Immunology of Host Defence Peptides: Beyond Antimicrobial Activity" (Nat Rev Immunol 2106 May; 16(5):321-34.

Antimicrobial activity vs. Gram-positive clinical isolates

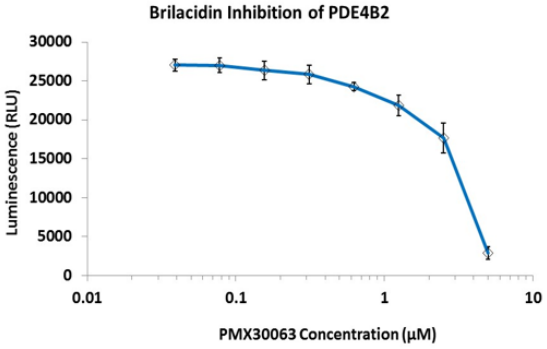
Organism Gram-positive	MIC (µg/ml) 2 - 3 isolates/organism				
	PMX30016	PMX30063	Linezolid	Vancomycin	Ceftazidime
Entero. faecalis	2	1	1 - 2	1	>64
Entero. faecium (VRE)	1	1	1 – 2	>128	>64
Staph. aureus (MRSA)	0.5	0.5 - 1	1 – 2	0.5 – 1	32
Staph. epidermidis	0.5	0.25 - 0.5	0.5 - 1	2	16 – 32
Staph. saprophyticus	0.5	0.25 - 0.5	1 - 2	1 – 2	32 - >64
Staph. spp. (coagulase -)	0.5	0.25 - 0.5	1	1 – 2	16 – 32
Strept. agalactiae	2 – 4	2	1	0.5	0.5
Strept. pneumoniae	8	4 - 8	1	0.5	0.25
Strept. pyogenes	1 – 2	1 – 4	1	0.5	0.12
Strept. viridians	8 - 16	2 – 8	1	0.5 - 1	0.5 - 4

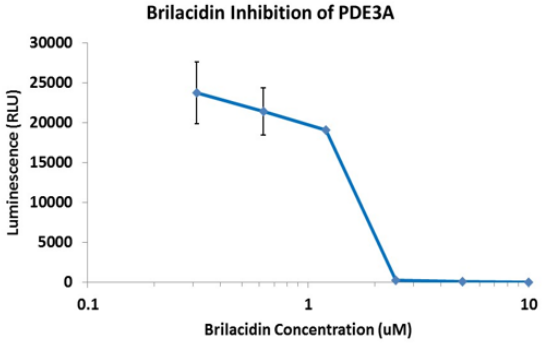
Antimicrobial activity vs. Gram-negative clinical isolates

Organism Gram-negative	MIC (µg/ml) (2 - 3 isolates/organism)				
	PMX30016	PMX30063	Ceftazidime	Linezolid	Vancomycin
Citrobacter freundi	4	2 - 4	0.25 – 2	>16	>128
Citrobacter koseri	2 – 4	1 - 2	0.12 – 0.25	>16	>128
Enterobacter cloacae	2	0.5 - 4	0.25	>16	>128
Escherichia coli	2	1 - 2	0.06	>16	>128
Klebsiella oxytoca	2 – 4	2 - 8	0.06 – 0.12	>16	>128
Klebsiella pneumoniae	2 – 4	1 - 2	0.06 – 0.12	>16	>128
Morganella morganii	>64	2 - >64	2 – 16	>16	>128
Proteus mirabilis	64	64 - >64	0.03 - 0.06	>16	>128
Proteus vulgaris	8 – 64	64 - >64	0.12	>16	>128
Providencia stuartii	4 – 16	16 - 64	0.12 – 64	>16	>128
Acinetobacter spp.	2 – 16	4	2 – 64	>16	128 - >128
Pseudomonas aeruginosa	8	32	1 – 8	>16	>128
Serratia marcescens	16 – 32	32	0.12 – 0.25	>16	>128
Stenotrophomonas maltophilia	4 – 64	8 - >64	4 – 8	>16	32 - 128
Haemophilus influenzae	8	4 - 8	0.06 – 0.12	16 - >16	128

Brilacidin

Inhibition of PDE4B2 and PDE3A





Phosphodiesterase inhibition assays demonstrated that Brilacidin inhibits both PDE4B2 (IC_{50} of 2.5 ± 0.21µM; n=5) and PDE3A (IC_{50} of 1.5 ± 0.2µM; n=4) in a dose dependent manner.

The PDE-Glo phosphodiesterase assay was performed using 8ng of PDE4B2 and 2.75ng of PDE3A, respectively, with 1µM cAMP substrate and indicated amount of Brilacidin.

Brilacidin and PDE4B2 and PDE3A, respectively, were mixed and pre-incubated at room temperature for 15 minutes. Substrate was added and the reaction was incubated for 7 minutes at room temperature.

Brilacidin Platform Potential

Gateway Concept Given Wide Range of HDP-M Therapeutic Activity

Innate Immunity

Direct Antimicrobial Activity

Bacterial

- ***ABSSSI (skin)****
- ***Bone and Joint***
- *DFIs*
- Respiratory
- Blood Stream
- STDs

Fungal

- Oral Candidiasis
- Disseminated Candidiasis
- Aspergillosis

Parasitic

- Malaria
- Sleeping Sickness
- Giardiasis

Viral

- Coronavirus (tbd)
- Influenza
- Herpes
- RSV

Barrier Function

GI Mucosa

- ***IBD: Ulcerative Proctitis (Distal Colitis)*****
- ***IBD: Ulcerative Colitis***
- ***Oral Mucositis***
- ***IBD: Crohn's***
- Irritable Bowel Syndrome
- GI-Acute Radiation Sickness
- Periodontitis

Respiratory Mucosa

- Cystic Fibrosis
- Asthma
- Chronic Bronchitis
- Lung-ARS
- Chronic Sinusitis

Skin/Eye

- ***Atopic Dermatitis***
- ***Acne***
- ***H. Suppurativa***
- Diabetic Ulcers
- Keratitis
- Burns/Abrasions

***ABSSSI** gateway for antibiotic opportunities

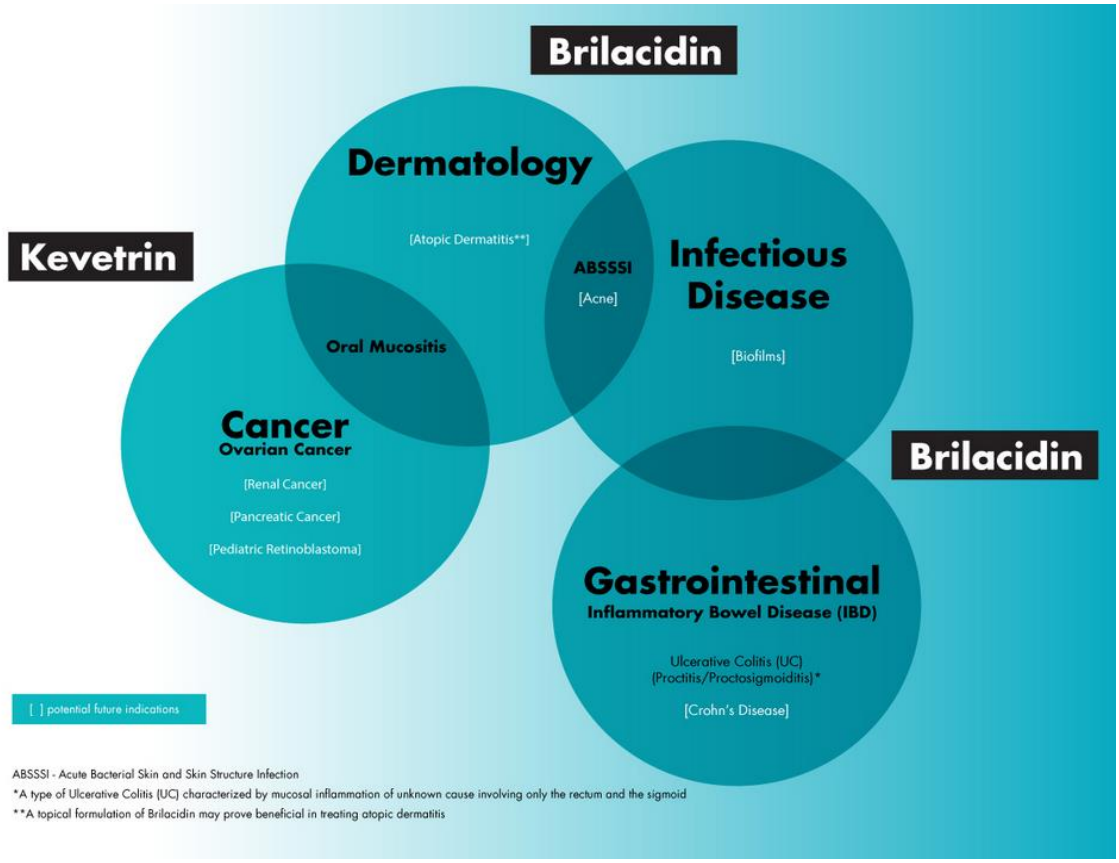
****Ulcerative Proctitis (Distal Colitis)** gateway for anti-inflammatory opportunities

Exceptionally Strong Pipeline, Novel Mechanisms of Action

Drug Candidate	Indication	Preclinical	Phase 1	Phase 2	Phase 3
Brilacidin	Oral Mucositis[1]				
	ABSSSI[2,3]				
	IBD: Ulcerative Colitis[4]				
	IBD: Crohn's Disease				
	IBD: UP/UPS[5]			ALFASIGMA	
	Atopic Dermatitis				
	Acne				
Kevetrin	Ovarian Cancer[6]				

Leveraging data from clinical studies in other indications to expedite development

[1] Awarded Fast Track Designation
[2] Acute Bacterial Skin and Skin Structure Infection
[3] Awarded Qualified Infectious Disease Product (QIDP) Designation (qualifies for Fast Track and Priority Review)
[4] Oral formulation mode of administration
[5] Inflammatory Bowel Disease: Ulcerative Proctitis/Ulcerative Proctosigmoiditis; licensed to Alfasigma S.p.A. (July 2019)
[6] Awarded Orphan Drug Designation



ABSSSI - Acute Bacterial Skin and Skin Structure Infection
*A type of Ulcerative Colitis (UC) characterized by mucosal inflammation of unknown cause involving only the rectum and the sigmoid
**A topical formulation of Brilacidin may prove beneficial in treating atopic dermatitis

Forward-Looking Statements: There is no assurance made or implied that testing of Brilacidin for any coronavirus will be conducted or successful. This informational document contains forward-looking statements made pursuant to the safe harbor provisions of the Private Securities Litigation Reform Act of 1995 including statements concerning future drug development plans, other statements regarding future product developments, and markets, including with respect to specific indications, and any other statements which are other than statements of historical fact. These statements involve risks, uncertainties and assumptions that could cause the Company's actual results and experience to differ materially from anticipated results and expectations expressed in these forward-looking statements. The Company has in some cases identified forward-looking statements by using words such as "anticipates," "believes," "hopes," "estimates," "looks," "expects," "plans," "intends," "goal," "potential," "may," "suggest," and similar expressions. Among other factors that could cause actual results to differ materially from those expressed in forward-looking statements are the Company's need for, and the availability of, substantial capital in the future to fund its operations and research and development; including the amount and timing of the sale of shares of common stock under securities purchase agreements; the fact that the Company's licensee(s) may not successfully complete pre-clinical or clinical testing and the Company will not receive milestone payments, or the fact that the Company's compounds may not successfully complete pre-clinical or clinical testing, or be granted regulatory approval to be sold and marketed in the United States or elsewhere. A more complete description of these risk factors is included in the Company's filings with the Securities and Exchange Commission. You should not place undue reliance on any forward-looking statements. The Company undertakes no obligation to release publicly the results of any revisions to any such forward-looking statements that may be made to reflect events or circumstances after the date of this press release or to reflect the occurrence of unanticipated events, except as required by applicable law or regulation.