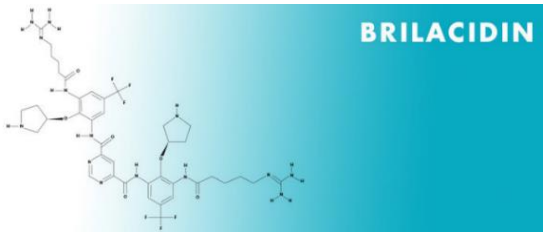
Brilacidin

First-in-Class Defensin-Mimetic Drug Candidate

Mechanism of Action, Pre/Clinical Data and Academic Literature Supporting the Development of Brilacidin as a Potential Novel Coronavirus (COVID-19) Treatment

April 20, 2020



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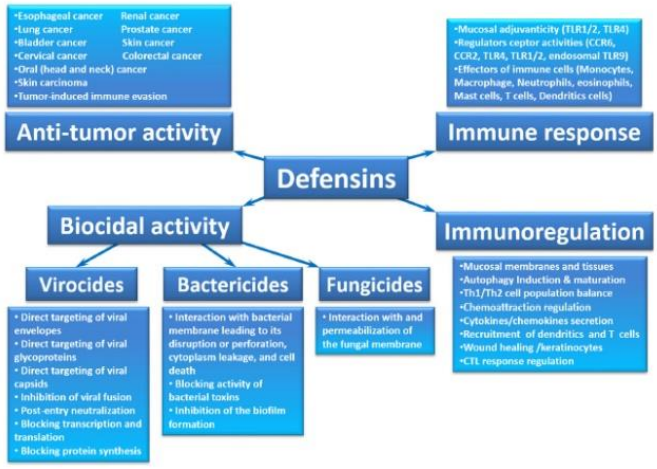
I. Brilacidin: Background Information

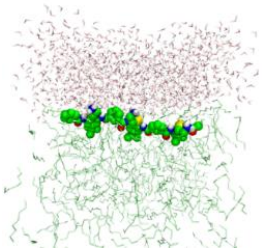
Brilacidin (PMX-30063) is Innovation Pharmaceutical's lead Host Defense Protein (HDP)/Defensin-Mimetic drug candidate targeting SARS-CoV-2, the virus responsible for COVID-19. Laboratory testing conducted at a U.S.-based Regional Biocontainment Laboratory (RBL) supports Brilacidin's antiviral activity in directly inhibiting SARS-CoV-2 in cell-based assays. Additional pre-clinical and clinical data support Brilacidin's therapeutic potential to inhibit the production of IL-6, IL-1β, TNF-α and other pro-inflammatory cytokines and chemokines (e.g., MCP-1), identified as central drivers in the worsening prognoses of COVID-19 patients. Brilacidin's antimicrobial properties might also help in fighting secondary bacterial infections, which can co-present in up to 20 percent of COVID-19 patients. Collectively, **these data support Brilacidin as a promising and unique—3 in 1 combination: antiviral, immune/anti-inflammatory, and antimicrobial—anti-COVID-19 drug candidate**. Additional drug delivery work (e.g., developed as an inhalant) might complement Brilacidin's anti-COVID-19 therapeutic potential. Brilacidin has been tested in multiple Phase 2 human trials for other clinical indications, providing an established safety and efficacy profile, thereby potentially enabling it to help confront the worldwide coronavirus crisis.

Exhibiting antiviral, immunomodulatory/anti-inflammatory and antimicrobial properties, Brilacidin has shown therapeutic benefit in successful Phase 2 clinical trials (*see Section V for summary safety and efficacy data*), including:

- **Acute Bacterial Skin and Skin Structure Infection** (ABSSSI) (FDA Qualified Infectious Disease Product, QIDP): Phase 2b (NCT02052388), *intravenous delivery*
- **Inflammatory Bowel Disease** (IBD): Phase 2 Proof-of-Concept in Ulcerative Proctitis/Ulcerative Proctosigmoiditis (UP/UPS), *enema formulation*; currently being developed as an *oral tablet* in Ulcerative Colitis (UC), Phase 2 planning
- **Oral Mucositis** (OM) (FDA Fast Track): Phase 2 (NCT02324335), *oral rinse delivery*; Phase 3 planning

Referred to as the "Swiss Army Knife" of the human body, defensins are small antimicrobial peptides (AMPs) expressed widely in the animal kingdom that serve as the first line of defense against pathogenic invasion of the body. Defensin-based therapeutics—of which Brilacidin is the leading example, and the most advanced drug candidate among this class in clinical testing—represent an attractive possible intervention to combat the coronavirus given their innate and multifaceted immune functions. Defensins exhibit a number of distinct and favorable therapeutic characteristics, including among others: *immunomodulatory, antiviral, anti-inflammatory and antimicrobial*.





Brilacidin, a fully synthetic non-peptidic mimetic of defensins, was computationally designed *de novo* to be smaller (1/10th the size), more stable, more potent (by a 100-fold), more selective (by a 1000-fold), and more easily manufactured, than natural defensins, so as to overcome many of the shortcomings (e.g., degradation, toxicity, lack of efficacy, malabsorption, cost to produce, etc.) that have complicated their clinical development. Brilacidin has shown tailored exposure and efficacy across multiple clinical indications.

II. Brilacidin: Two Primary Mechanisms of Action

HDPs/Defensins are Small Antimicrobial Peptides

- Expressed widely in the animal kingdom
- Evolutionarily conserved
- Produced in skin, mucosal surfaces, neutrophils
- Target membranes (primary MOA)
- Modulate immune response (primary MOA)
- Properties act in synergistic fashion

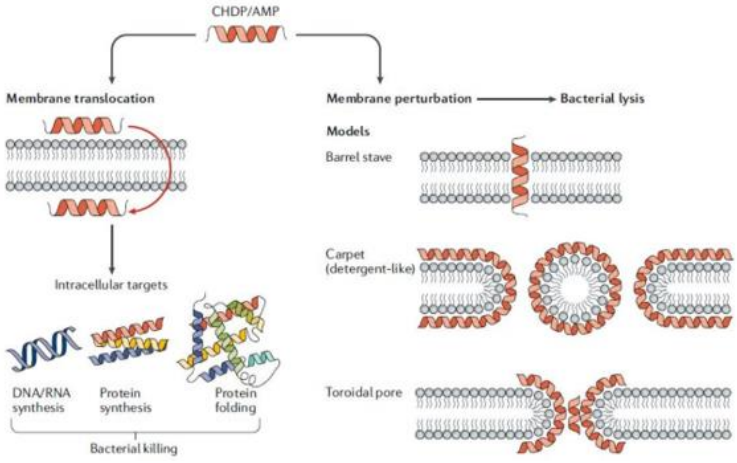
Serve as First Line of Defense Against Pathogens

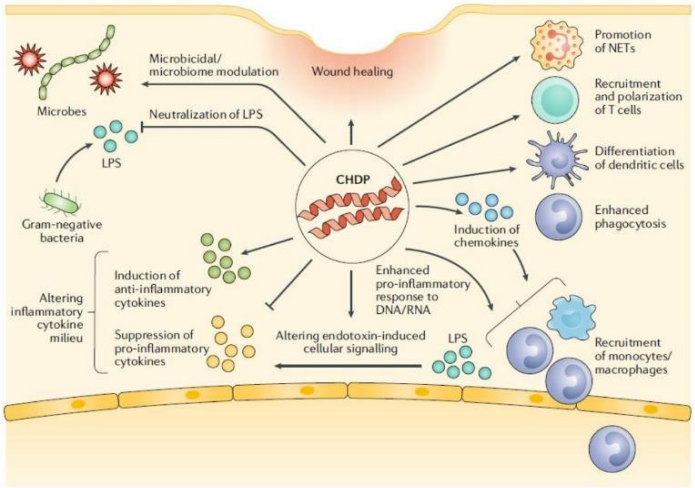
- Part of innate and adaptive immunity
- Maintenance of epithelial barrier function
- Regulate microbiota
- Immunomodulatory/Anti-Inflammatory properties
- Antimicrobial properties
- Antiviral properties

Brilacidin

1) Membrane Disruption MOA
Amphiphilic Structure
Cationic/Hydrophobic

2) Immunomodulatory MOA
cAMP Pathway
PDE4/PDE3 Inhibition





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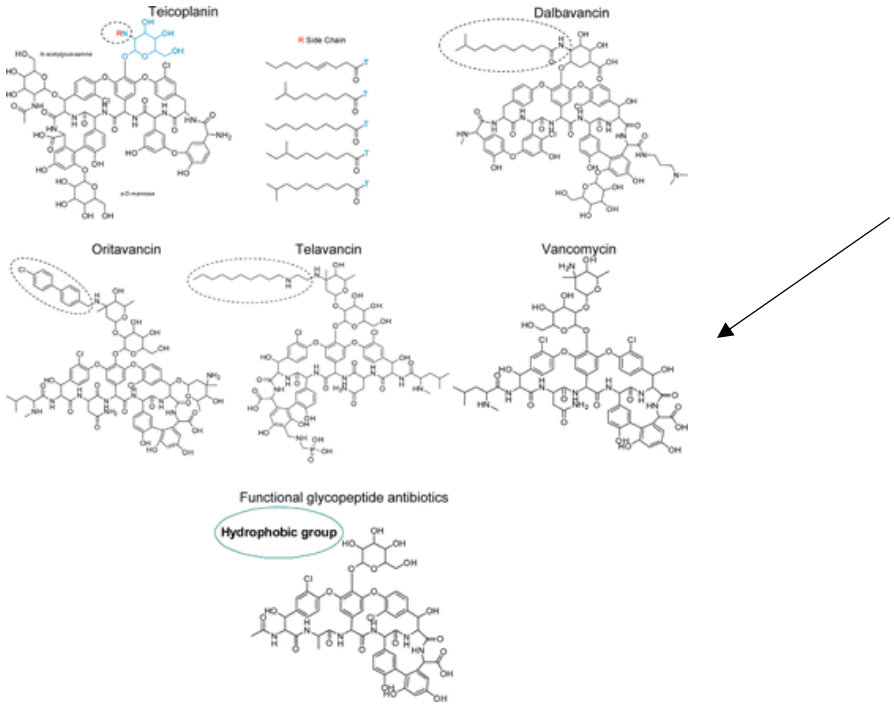
Membrane Disruption Mechanism of Action

As to bacterial/viral invasion, AMP/defensins and mimetics, like Brilacidin—via an amphiphilic topology: both positively charged and hydrophobic (water-hating, lipid-loving) properties—target the structural plasticity/thermodynamic instability of invading toxins, thus increasing their susceptibility to proteolysis and degradation. Other intra-cellular mechanisms beyond membrane-active properties—a capacity for acting on numerous targets and by means of a variety of mechanisms—also play an important role in contributing to the overall antibacterial/antiviral efficacy of AMP/defensin-based therapies, via what's been referred to as a "multiple-hit model." For additional detailed information on the biophysics of this process, including mechanistic studies conducted on Brilacidin, refer to the two articles linked below:

- Mensa B, et al. "Comparative Mechanistic Studies of Brilacidin, Daptomycin, and the Antimicrobial Peptide LL16." *Antimicrob Agents Chemother*. 2014 Sep;58(9):5136-45. doi: 10.1128/AAC.02955-14. Epub 2014 Jun 16.
- Mensa B, et al. "Antibacterial Mechanism of Action of Arylamide Foldamers." *Antimicrob Agents Chemother*. 2011 Nov;55(11):5043-53. doi: 10.1128/AAC.05009-11. Epub 2011 Aug 15.

Teicoplanin-based glycopeptide antibiotics (Dalbavancin, Telavancin, Oritavancin) have been shown to inhibit viral entry of MERS and SARS/SARS-CoV-2 viruses. Interestingly, Vancomycin did not. Researchers attributed this lack of anti-MERs and anti-SARS efficacy due to the drug not having a hydrophobic property[1], again, which Brilacidin possesses. In the 2014 Mensa et al article cited above, the authors reference Brilacidin showing certain membrane-lytic properties similar to those of Telavancin.[2]



Hydrophobic group absent in Vancomycin, theorized as to why it showed no antiviral activity against MERS and SARS/SARS-CoV-2 viruses in pre-clinical studies unlike other glycopeptide antibiotics

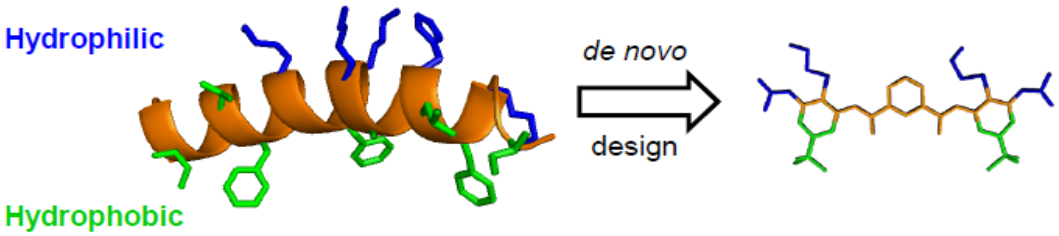
Brilacidin exhibits hydrophobic properties

[1] See: Badani H, et al. "Peptide Entry Inhibitors of Enveloped Viruses: The Importance of Interfacial Hydrophobicity." *Biochim Biophys Acta.* 2014 Sep;1838(9):2180-97. doi: 10.1016/j.bbamem.2014.04.015. Epub 2014 Apr 26.

[2] "Transcriptional response studies have shown a strong induction of the VraSR regulon in response to telavancin treatment, as observed for brilacidin treatment and as expected from a cell wall synthesis inhibitor."

Fully synthetic non-peptidic mimetic drugs, such as Brilacidin, an arylamide foldamer, are considered attractive therapeutic candidates given favorable characteristics, including being: (i) highly selective (potent against target while leaving host cells unaffected); (ii) not prone to resistance mechanisms; (iii) relatively easy to produce at low costs; (iv) and stable during storage or upon administration.

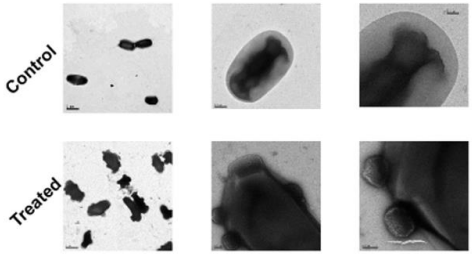
This biocomputational aspect of Brilacidin's development (de novo design[3]) has resulted in the drug candidate having much better exposure and efficacy in terms of its pharmacokinetics, contributing to broad and robust properties.



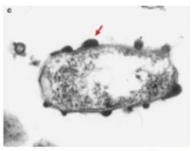
Brilacidin: Primary Mechanism of Action #1 (Membrane Disruption)

Directly Disrupts Microbial Membranes/Viral Envelopes… Leading to Bacterial Cell Death/Viral Non-Viability*

Brilacidin functions as an antimicrobial/antiviral, piercing the cell walls of bacteria (*bactericidal*) and envelopes of viruses (*virucidal*)



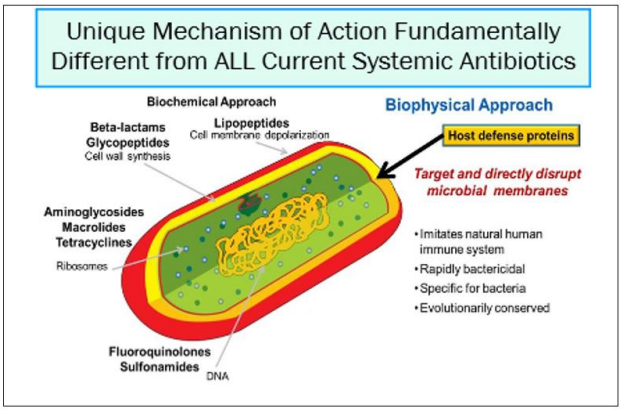
TEM of *P. aeruginosa* on SMAP29 (3 hrs)



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.

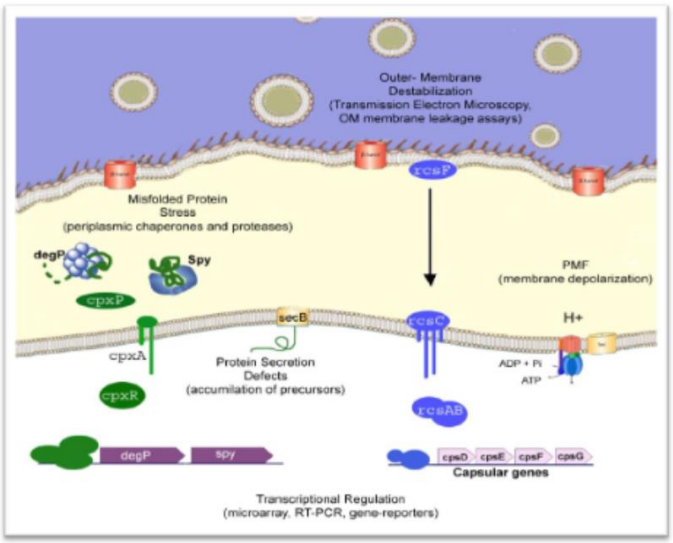
**Current theory re viruses; further mechanistic research underway*

[3] See: Scott RW, et al. "De Novo Designed Synthetic Mimics of Antimicrobial Peptides." *Curr Opin Biotechnol*. 2008 Dec; 19(6): 620–627; Tew GW, et al. "De Novo Design of Antimicrobial Polymers, Foldamers, and Small Molecules: From Discovery to Practical Applications." *Acc Chem Res.* 2010 Jan 19;43(1):30-9; Lienkam K, et al. "Antibacterial Peptidomimetics: Polymeric Synthetic Mimics of Antimicrobial Peptides. (pdf) In: Abe A, Kausch HH, Möller M, Pasch H (eds) "Polymer Composites – Polyolefin Fractionation – Polymeric Peptidomimetics – Collagens." *Advances in Polymer Science*, vol 251. Springer, Berlin, Heidelberg.

Current view on MOA for optimized arylamides

in collaboration with W. DeGrado (UCSF)

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

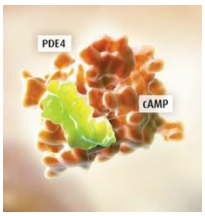


Advantages: Mimetic Approach

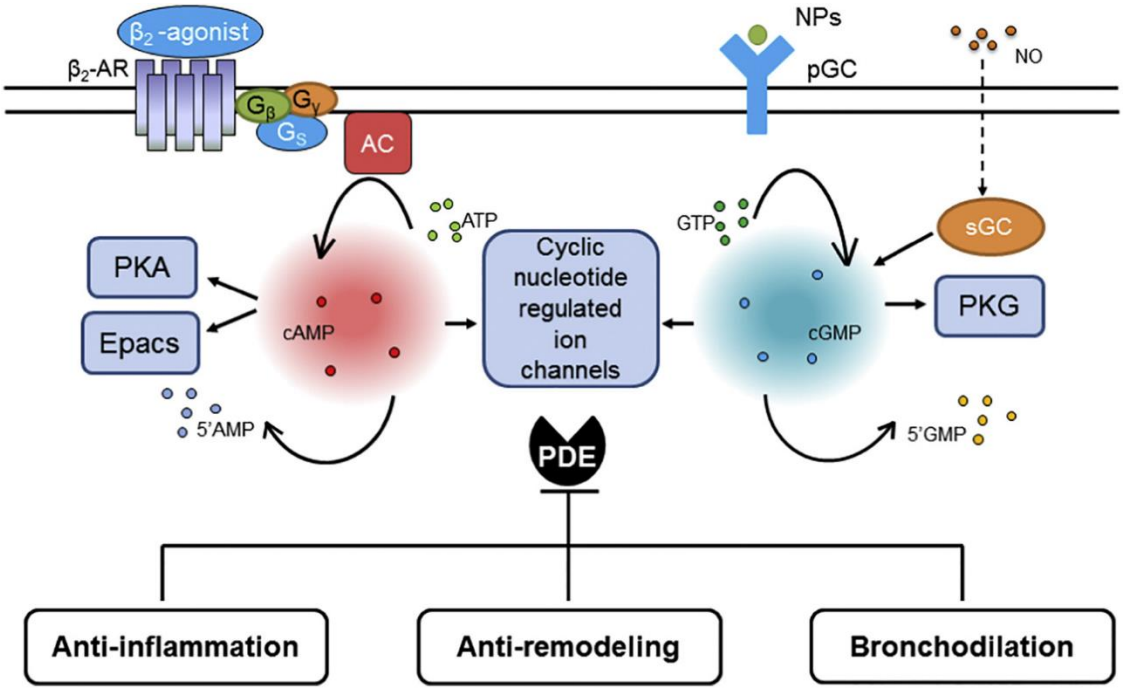
- **Narrow and broad-spectrum antimicrobial agents have been produced**
  - 0.5 to 2 µg/ml MICs vs Gram-positives
  - 0.5 to 8 µg/ml MICs vs Gram-negatives
- **Wide selectivity for bacteria over mammalian cells**
  - Significant improvements in cytotoxicity versus HDPs
  - >100 to 1,000 fold selectivities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

Immunomodulatory Mechanism of Action

Brilacidin, through its modulation of the cyclic adenosine monophosphate (cAMP) pathway, has been shown to be a potent regulator of immune response.



Pre-clinical studies have demonstrated that Brilacidin inhibits PDE4B2 and PDE3A *in vitro*, in a dose dependent manner. Brilacidin demonstrated similar IC50 values against both PDE4 (biochemical) and cytokine release in cell-based assays, suggesting Brilacidin has good cell membrane permeability. Localized clinical administration enables Brilacidin concentrations that markedly exceed *in vitro* IC50 values, and, consequently, provides for increased concentrations of cAMP. Drugs that elevate intracellular cAMP levels reduce pro-inflammatory mediators and increase anti-inflammatory factors in numerous immune cells. The effect of Brilacidin's ability to modulate cAMP levels—complementing other aspects of its mechanisms of action—supports its potential to treat a number of chronic, autoimmune and inflammatory diseases related to issues of innate immunity, such as: Inflammatory Bowel Disease, Atopic Dermatitis, and potentially COVID-19.



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Brilacidin, as an inhibitor of 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 COVID-19. Defensins play a key role in pulmonary and mucosal host defense. In March 2020, a Deep Learning AI program[4] was used by researchers to screen (pdf) almost 5,000 approved drugs and identified Roflumilast, a PDE4 inhibitor, like Brilacidin, as one of only ten most promising anti-SARS-CoV-2 drug candidates.

[4] See also the March 23 launch of the COVID-19 High Performance Computing Consortium; also see Gordon D, et al. "A SARS-CoV-2-Human Protein-Protein Interaction Map Reveals Drug Targets and Potential Drug-Repurposing" (March 27, 2020) (BioRxiv).

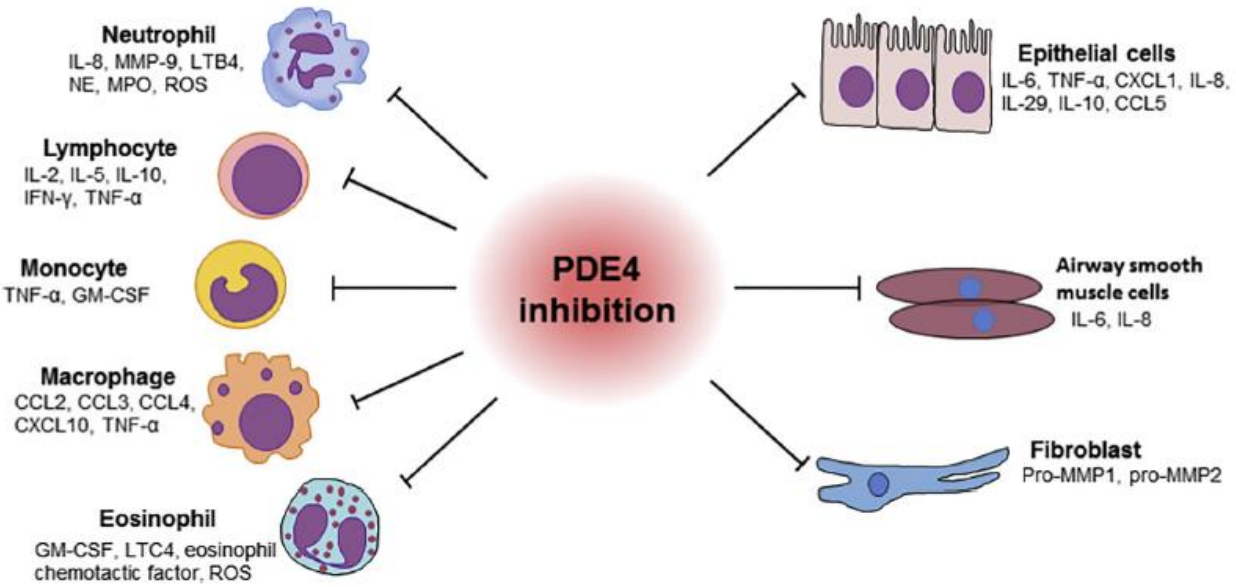
Additional Commentary on Relevance to COVID-19

In normal physiology, on initial encounter with viruses, resident alveolar macrophages become activated and secrete pro-inflammatory cytokines and chemokines, resulting in the recruitment of neutrophils, macrophages, and lymphocytes. A pro-inflammatory positive feedback loop between the tissue-resident macrophages and recruited cells amplifies the immune response, and promotes further immune cell recruitment. As the infection is cleared, a second wave of anti-inflammatory cytokines is produced to dampen the immune response to avoid immune-mediated damage.

This cycle of immune response amplification followed by response attenuation has been demonstrated to be less robustly regulated in older patients. The innate immune response to tissue damage caused by the novel coronavirus could lead to Acute Respiratory Distress Syndrome (ARDS) in which respiratory failure is characterized by rapid onset of widespread inflammation in the lungs and subsequent death. The level of inflammatory cytokines is highly expressed in the lungs of hospitalized COVID-19 patients. Key molecular players include IL-6, TNF-α and IL-1. Recent autopsies have confirmed that the lungs are filled with clear liquid jelly, which is similar to that caused by hyaluronan associated with ARDS. Hyaluronan increases MMP9.

Brilacidin has been shown to inhibit IL-6, as well as other pro-inflammatory cytokines and chemokines (e.g., TNF-α, IL-1β, IL-6, IL-8, MIP2-α, MCP-1, MMP-9, and CINC-3), thereby positioning the drug as a promising intravenous treatment for COVID-19 patients. Given the redundancy of cytokine actions, targeting a single cytokine may have only limited effects on the complex inflammatory process.

Brilacidin, through its modulation of the cyclic adenosine monophosphate (cAMP) pathway acting to inhibit PDE4/PDE3, has the ability to affect multiple immune-related steps and regulate multiple cytokines and chemokines to mitigate severity of complex inflammatory processes, including respiratory distress implicated in COVID-19.



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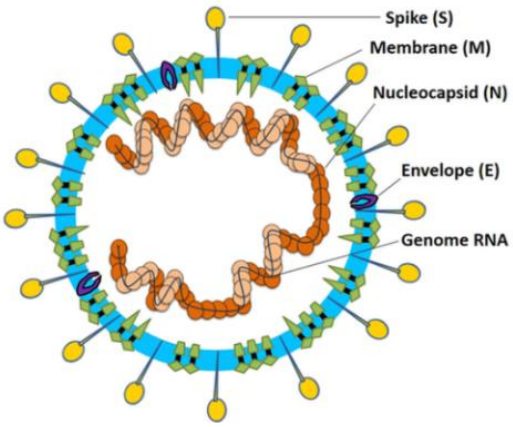
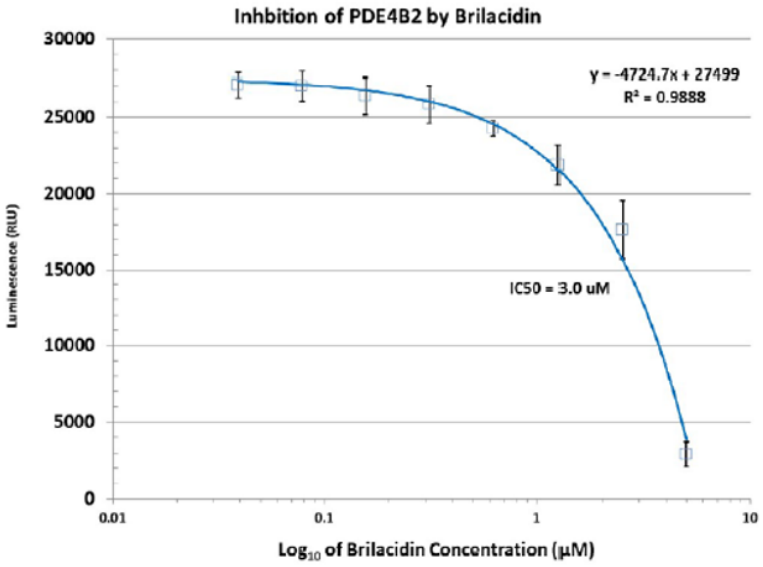
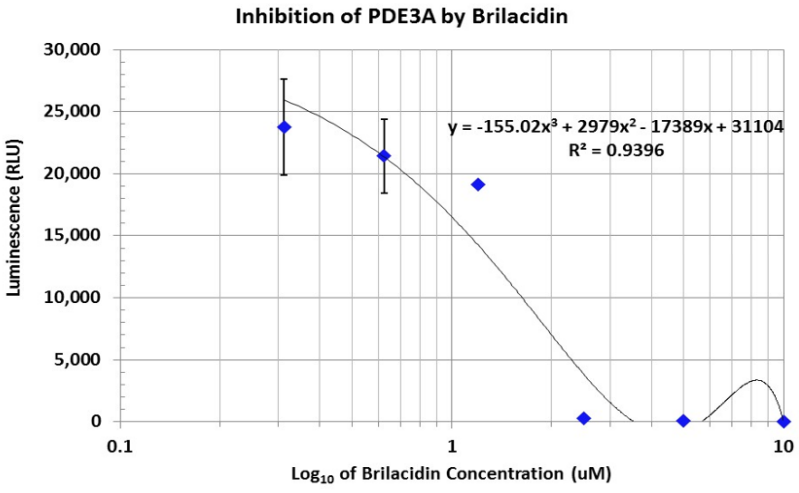
The three Review Articles on coronaviruses, linked below, also suggest immunomodulators, like Brilacidin, might be beneficial therapeutic options for treating coronaviruses, potentially acting synergistically when combined with other antiviral drugs.

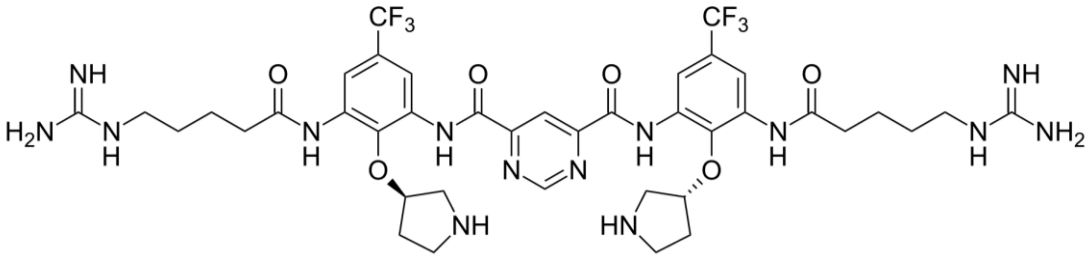


- Li G, et al. "Coronavirus Infections and Immune Responses." *Journal of Medical Virology* (Vol 92, Issue 4): 424-432. doi.org/10.1002/jmv.25685 (January 25, 2020).
- Li G, and E De Clercq. "Therapeutic Options for the 2019 Novel Coronavirus (2019-nCoV)." *Nat Rev Drug Discov*. 2020 Mar;19(3):149-150. doi: 10.1038/d41573-020-00016-0.
- 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.





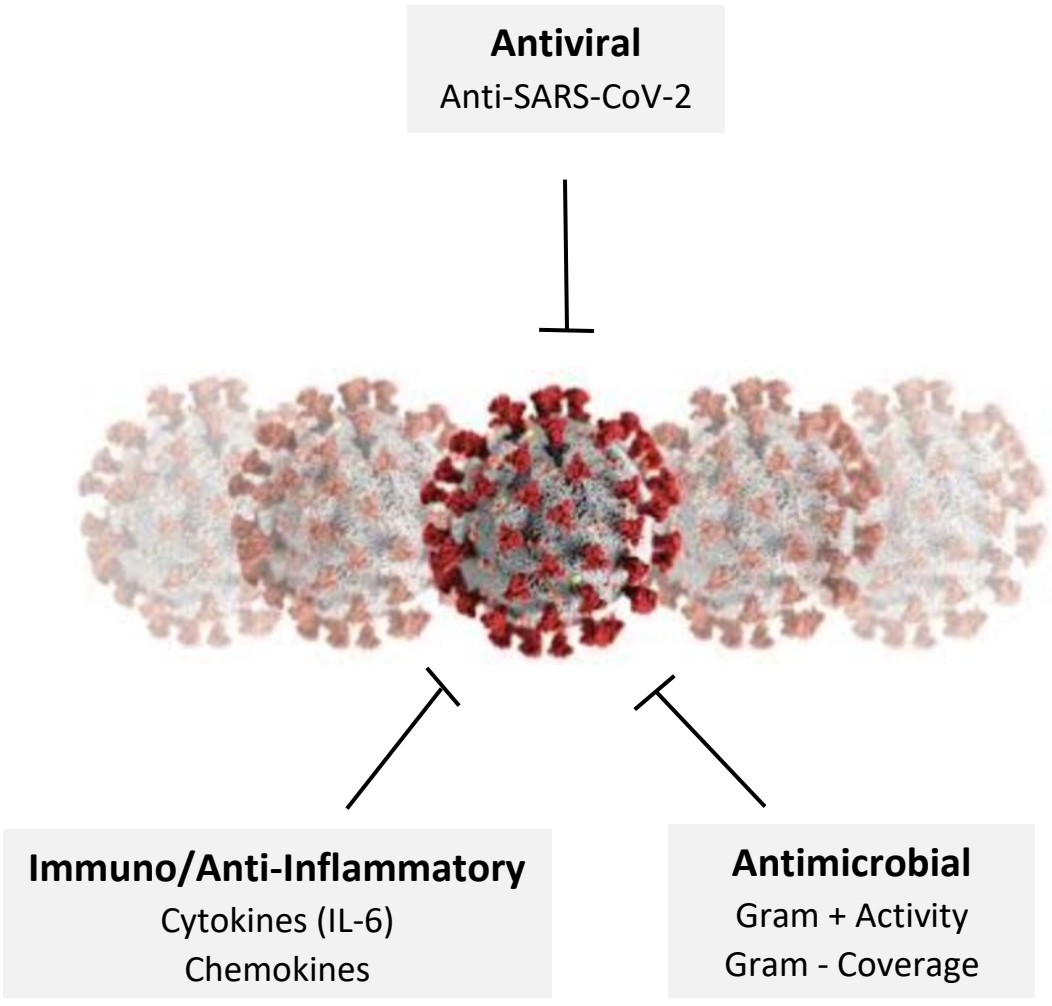
III. Brilacidin: Several Complementary Ways of Targeting COVID-19



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

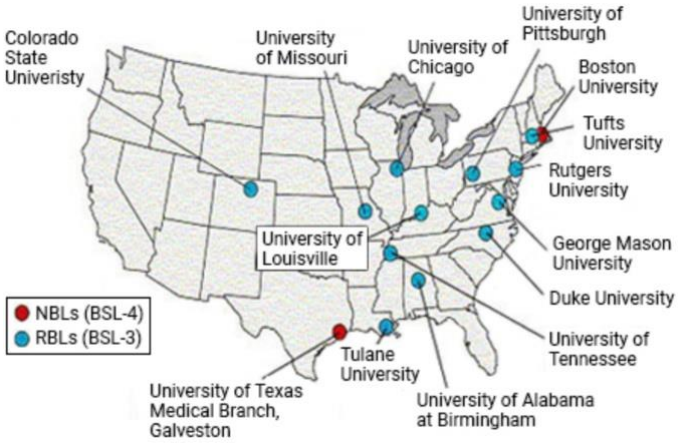
Molar Mass 936.9 g/mol

3-in-1 Combination of Therapeutic Properties



Antiviral

The antiviral testing of Brilacidin was conducted at one of the U.S. Regional Biocontainment Laboratories (RBLs). Based on a recent review article, few compounds show activity against SARS-CoV-2, an enveloped virus.



VERO cells, a monkey kidney cell line commonly used to screen small molecule inhibitors of viruses, were used to test whether Brilacidin inhibits SARS-CoV-2. Cells were pretreated with Brilacidin at increasing concentrations (at 2 µM and at 10 µM) for two hours prior to the infection. Cells treated with the vehicle alone (Dimethyl sulfoxide or DMSO) were maintained alongside, as controls.

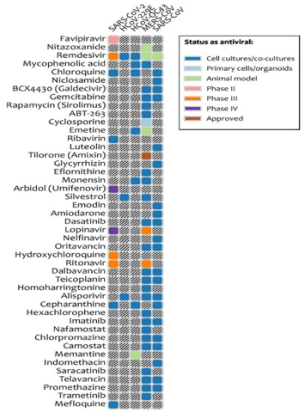
At 16 hours post-infection (16hpi), researchers observed a dose-dependent reduction in the SARS-CoV-2 infectious viral titers from the Brilacidin treated cells as compared to the vehicle-alone control, as is shown in the graphic below. (The higher number of asterisks denote higher statistical significance compared to control.) Additional laboratory testing of Brilacidin against SARS-CoV-2, the novel coronavirus responsible for COVID-19, is to commence shortly. This is based on recommendations made by the lead researcher at the RBL who performed the preliminary testing of Brilacidin, characterizing the results as "extremely encouraging." Immediate next steps include conducting studies in human lung cells, exploring dosing/treatment windows and evaluating the drug's effect on the viral envelope.

Brilacidin Antiviral Properties

Few Compounds Show Antiviral Activity Against SARS-CoV-2; Early Efficacy Signal (Direct Inhibition)

Anti-Coronavirus Compounds

By Stage of Development



Source: Andersen P, et al. "Discovery and Development of Safe-in-Man Broad-Spectrum Antiviral Agents." *Int J Infect Dis.* 2020 Feb 17;93:268-276. doi: 10.1016/j.ijid.2020.02.018.

Research Conducted at U.S. Regional Biocontainment Lab

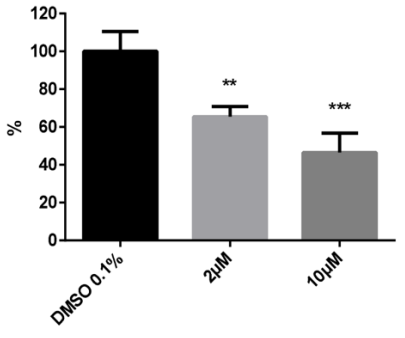
Experiment

- 16 Hour Post Infection (cellular assay)
- In Vero Cells (monkey kidney line)
- Increasing concentration (2 and 10 µM) prior to infection
- Inhibitory dose-dependent effect observed as a measure of viral titers
- Activity supports further testing

Next Steps

- Conduct testing in lung cells
- Assess further
  - Max tolerated dose
  - Multiple dose potential
  - Treatment windows
- Mechanistic studies
  - Effect on viral envelope
  - Antiviral/Anti-inflammatory profile
  - Combination with other drugs

Efficacy of Brilacidin (16hpi) against SARS-CoV-2 in VERO Cells



The higher number of asterisks denote higher statistical significance compared to control

See: accompanying document (experimental writeup):
"Antiviral Activity of Brilacidin in the Context of SARS-CoV-2 Infection"

Of note, two recent journal articles, summarized and linked below, also support Brilacidin as a promising potential inhibitor of SARS-CoV-2.

- Cavasotto C, et al (2020). "In Silico Drug Repurposing for COVID-19: Targeting SARS-CoV-2 Proteins through Docking and Quantum Mechanical Scoring" (pdf). *ChemRxiv.* Preprint. April 12, 2020.

  Based on a molecular docking-based virtual screening of 11,552 structurally diverse compounds either already FDA-approved or in clinical testing—Brilacidin, due to its unique molecular properties, was identified as one of the most promising potential inhibitors of SARS-CoV-2 by targeting the spike 1 glycoprotein (S1) (see image below). The researchers concluded: "Clearly, these compounds should be further evaluated in experimental assays and clinical trials to confirm their actual activity against the disease."

Table 1: Potential inhibitors of SARS-CoV-2 M^{pro} from existing drugs and compounds undergoing clinical trials (DB, DrugBank; CH, ChEMBL).

Drug name	Drug ID	Pharmacological function
Felypressin	DB00093	Vasoconstrictor
Angiotensinamide	DB13517	Vasoconstrictor
Briladicin	CH2219413	Head and neck neoplasms
Ritonavir	DB00503	HIV-protease inhibitor
Samatasvir	CH3039519	Hepatitis C infection
Indinavir	DB00224	HIV-protease inhibitor
CR665	DB05155	κ-opioid receptor agonists
Lopinavir	CH729	HIV-protease inhibitor
	DB02747	N/A
	DB04692	N/A
	DB04722	N/A
	DB03311	N/A

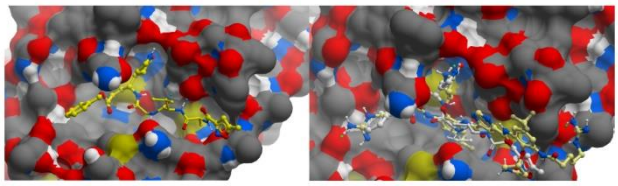
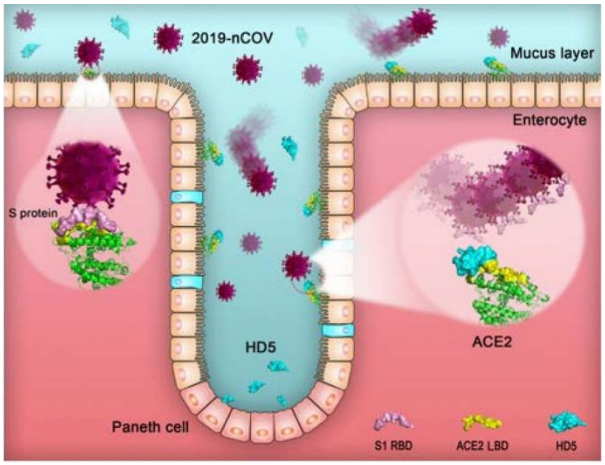


Figure 1: Ritonavir (left panel) and Brilacidin (right panel) docked within the binding site of SARS-CoV-2 M^{pro}. The receptor surface is colored as: red, oxygens; blue, nitrogen; white, polar hydrogen; grey, non-polar atoms. On the right panel, native ligand N3 (6LU7) is also displayed with white carbon atoms superimposed to Brilacidin (yellow carbons). Figure prepared with ICM (Molsoft LLC, San Diego, CA).

- Wang C, et al (2020). "Lectin-like Intestinal Defensin Inhibits 2019-nCoV Spike binding to ACE2" (pdf). *bioRxiv* 2020.03.29.013490. March 31, 2020.

  In this article, human defensin 5 (HD5) was shown to lower the ability of the novel coronavirus (nCoV) spike 1 glycoprotein (S1) to bind to angiotensin-converting enzyme-2 (ACE2), a receptor that nCoV uses to mediate entry into host cells. Researchers currently evaluating Brilacidin's anti-SARS-CoV-2 therapeutic potential theorize that Brilacidin, as a defensin-mimetic, may show similar nCoV inhibitory activity due to this particular mechanism of action, in addition to potentially disrupting the nCOV viral envelope directly due to the Brilacidin's hydrophobic properties.



Schematic illustration of the Paneth cell-mediated host defense against 2019-nCoV.

Human Paneth cells specifically secrete a lectin-like peptide named HD5, the most abundant α defensin in intestine. HD5 intensively binds and blocks epithelial ACE2, which is located on the brush border of enterocytes and is a recognized receptor for 2019-nCoV spike, thus weakening the viral adhesion and exerting a protective effect.

Immuno/Anti-Inflammatory

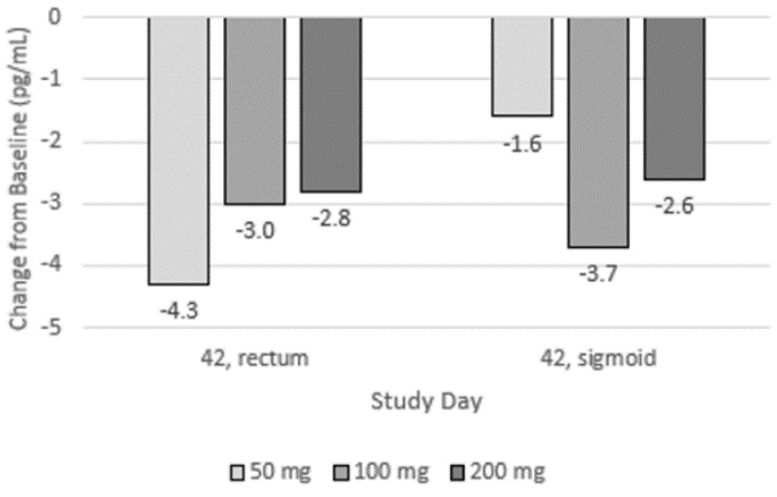
An excessive immune response to fight the novel coronavirus (triggering a "cytokine storm") is theorized to play an important role in COVID-19 disease severity, which can lead to Acute Respiratory Distress Syndrome (ARDS)—a serious respiratory complication necessitating mechanical ventilation and leading cause of death among COVID-19 patients. Scientists at the University of Science and Technology of China (USCT) have identified interleukin 6 (IL-6), a pro-inflammatory cytokine, as the "main culprit" in the body's overreaction when trying to fend off the virus.

Brilacidin, through its modulation of the cyclic adenosine monophosphate (cAMP) pathway, has been shown to be a potent regulator of immune response. Brilacidin has been shown to inhibit IL-6, as well as other pro-inflammatory cytokines and chemokines (e.g., TNF-α, IL-1β, IL-6, IL-8, MIP2-α, MCP-1, MMP-9, and CINC-3), thereby positioning the drug as a potential promising treatment for COVID-19 patients.

Anti-inflammatory Nonclinical Studies: in vitro and ex vivo

Study Type	Test System	IPI Study Number	Brilacidin IC_{50}	Unit
Inhibition of human PDE4B2	in vitro PDE-Glo™ PDE assay	2014-05-29	3	μM
Inhibition of human PDE3A	in vitro PDE-Glo™ PDE assay	2014-07-18	1.8	μM
Inhibition of LPS-induced TNF-α release	Rat alveolar macrophage (NR8383) cells; ELISA assay	2014-08-21	442	nM
Inhibition of LPS-induced MMP-9 release	Rat alveolar macrophage (NR8383) cells; ELISA assay	2014-11-10	2.3	μM
Inhibition of LPS-induced MCP-1 release	Rat alveolar macrophage (NR8383) cells; ELISA assay	2014-11-13	750	nM
Inhibition of LPS-induced IL-6 release	Rat alveolar macrophage (NR8383) cells; ELISA assay	2014-12-03	274	nM
Inhibition of LPS-induced IL-1β release	Rat alveolar macrophage (NR8383) cells; ELISA assay	2015-02-10	702	nM
Inhibition of LPS-induced CINC-3 release	Rat alveolar macrophage (NR8383) cells; ELISA assay	2015-02-16	425	nM
Inhibition of LPS-induced TNF-α release	Human monocytic leukemia (THP-1) cells; ELISA assay	2016-07-21	23.4	μM
Inhibition of LPS-induced IL-8 release	Human monocytic leukemia (THP-1) cells; ELISA assay	2016-08-02	10.8	μM

IL-6 Human Tissue Biopsy at Day 42 (Phase 2 UP/UPS Trial)

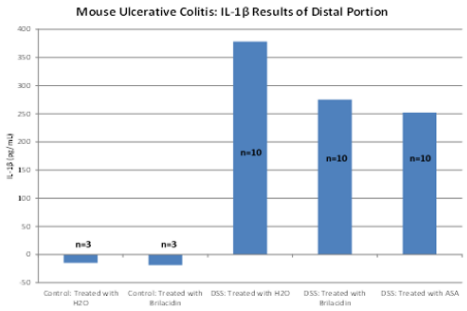


Brilacidin

Inhibition of IL-1ß, IL-6 (Mouse Model, Ulcerative Colitis)

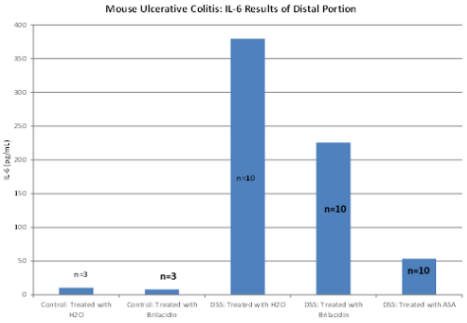
Brilacidin reduced the severity of Ulcerative Colitis (UC) induced by DSS administered through drinking water in mice. Brilacidin 400mg/kg were given intra-rectally once per day for 5 days. 5-amino salicylic acid (5-ASA) was used as a positive control. At the end of experiments, animals were anaesthetized. Their abdomens were and colons were washed directly with co-opened PBS (pH7) and immediately cut into small 1cm piece (distal portion) and transferred to liquid nitrogen container for further study. Frozen colon tissues were lysed and protein concentrations were measured and all samples were diluted to equal concentration. IL-6 and IL-1β were measured according to manufacturer's instruction.

Mouse Ulcerative Colitis: IL-1β



Effects of Brilacidin on DSS-induced IL-1β. The data were normalized to the total proteins. Levels of IL-1β levels presented as treated UC with Brilacidin (400mg/kg) relative to untreated UC. 5-ASA were used as positive control.

Mouse Ulcerative Colitis: IL-6



Effects of Brilacidin on DSS-induced IL-6. The data were normalized to the total proteins. Levels of IL-6 levels presented as treated UC with Brilacidin (400mg/kg) relative to untreated UC. 5-ASA were used as positive control.

Brilacidin

Inhibition of TNF-α, IL-1ß, IL-6

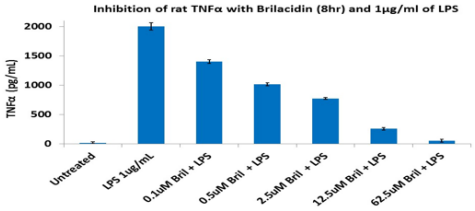


Fig. 1

Fig 1. Effects of Brilacidin on LPS-induced TNF-α release in NR8383 cells. Rat macrophages (NR8383) were pretreated with Brilacidin with concentrations shown for 45 minutes, followed by LPS (1µg/ml) treatment for 8 hrs. After 8 hrs, supernatants were collected for TNF-α measurement by ELISA.

Fig 2. Effects of Brilacidin on LPS-induced IL-6 release in NR8383 cells. Rat macrophages (NR8383) were pretreated with Brilacidin with concentrations shown for 45 minutes, followed by LPS (1µg/ml) treatment for 8 hrs. After 8 hrs supernatants were collected for IL-6 measurement by ELISA.

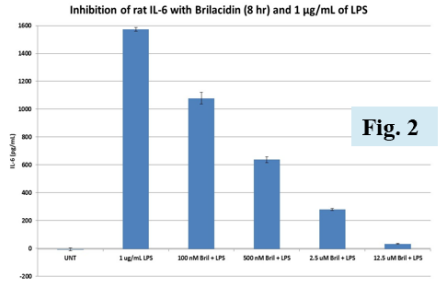


Fig. 2

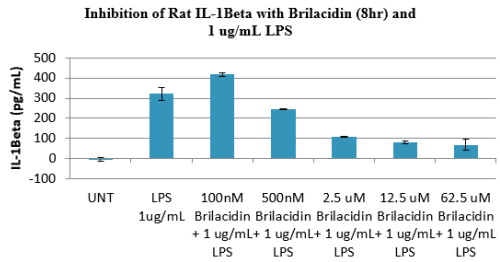
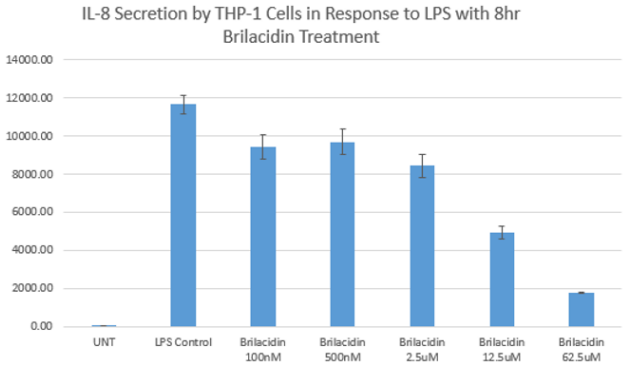


Fig. 3

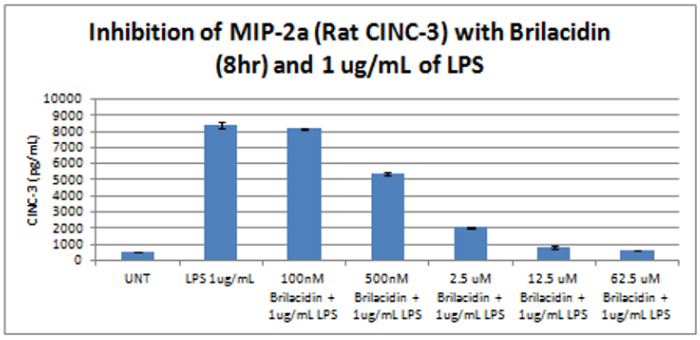
Fig 3. Effects of Brilacidin's on IL-1β production in rat macrophage cells pretreated with the compound. Brilacidin demonstrated a strong inhibition of IL-1β induction after LPS stimulation. There was more than 50% decrease in IL-1β production within 8 hrs of treatment at 2.5µM concentration of Brilacidin.

Brilacidin

Inhibition of IL-8, MIP2-α



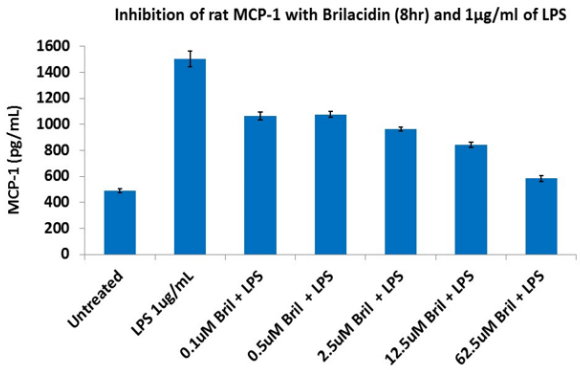
Effects of Brilacidin on LPS-induced IL-8 production in THP-1 cells. THP-1 cells were pretreated with Brilacidin with concentrations shown for 45 minutes, followed by LPS (1µg/ml) treatment for 8 hrs. After 8 hours, IL-8 concentrations were determined by ELISA using an immunoassay kit specific for human IL-8 (Thermo Fisher). Brilacidin inhibited the LPS-induced IL-8 production in THP-1 cells in a dose-dependent manner.



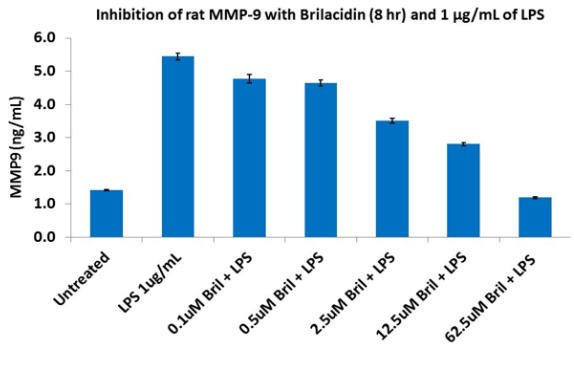
Effects of Brilacidin on LPS-induced MIP-2a (Rat CINC-3) release in NR8383 cells. Rat macrophages (NR8383) were pretreated with Brilacidin with concentrations shown for 45 minutes, followed by LPS (1µg/ml) treatment for 8 hrs. After 8 hrs, supernatants were collected for MIP-2a (Rat CINC-3) measurement by ELISA. A 65% decrease in CINC-3 levels at a 2.5µM concentration of Brilacidin was observed.

Brilacidin

Inhibition of MCP-1, MMP-9



Effects of Brilacidin on LPS-induced MCP-1 release in NR8383 cells. Rat macrophages (NR8383) were pretreated with Brilacidin with concentrations shown for 45 minutes, followed by LPS (1µg/ml) treatment for 8 hrs. After 8 hrs, supernatants were collected for MCP-1 measurement by ELISA.



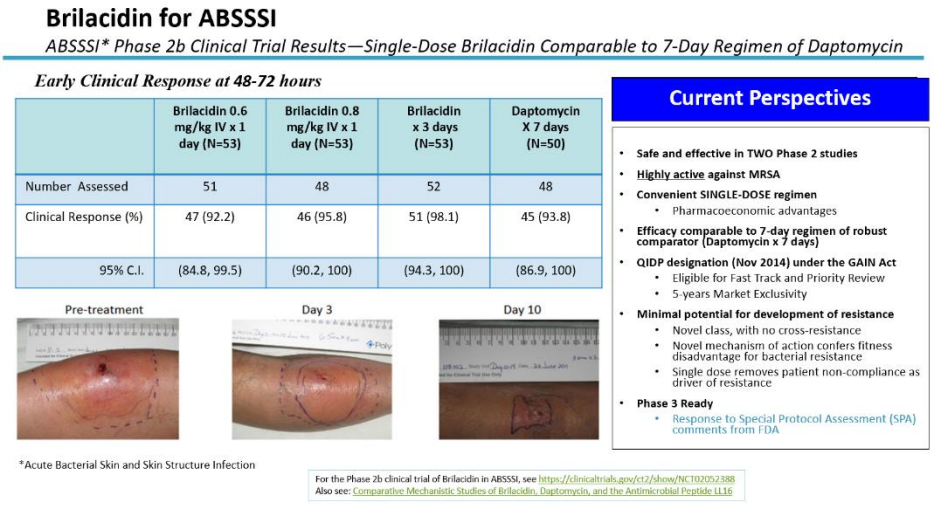
Effects of Brilacidin on LPS-induced MMP-9 release in NR8383 cells. Rat macrophages (NR8383) were pretreated with Brilacidin with concentrations shown for 45 minutes, followed by LPS (1µg/ml) treatment for 8 hrs. After 8 hrs, supernatants were collected for MCP-1 measurement by ELISA.

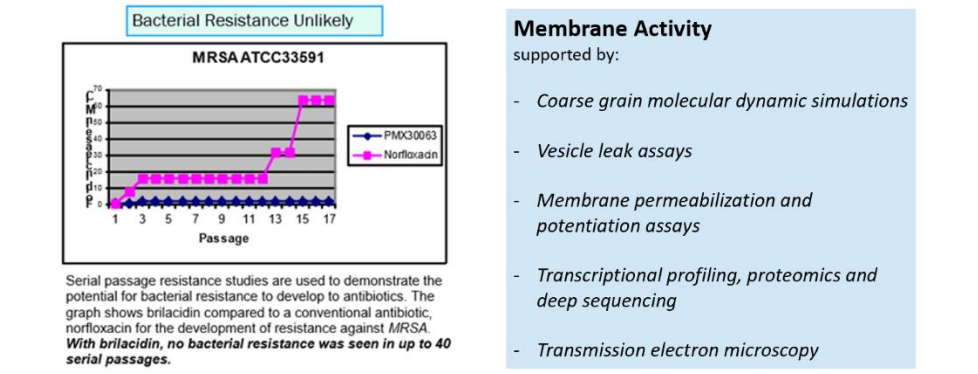
Antimicrobial

Even though a vast majority of COVID-19 patients are administered IV antibiotics, bacterial infections can co-present, in up to 15 percent of patients according to one study (even as high as 20 percent according to another study), with 50 percent of those who have died having had a secondary infection.

Brilacidin may help fight secondary infections among COVID-19 patients given its robust activity against opportunistic pathogenic bacteria, both gram positive and gram negative, including Methicillin-resistant Staphylococcus aureus (MRSA) and other drug-sensitive/drug-resistant bacteria. Julie L. Gerberding, Director of the CDC from 2002 to 2009, has called antibiotic resistance "an even larger threat lurking behind" the current COVID-19 pandemic.[5]

Bacterial superinfections, tied to secondary bacterial pneumonia caused by *Staphylococcus aureus* or *Streptococcus pneumoniae*, accompany flu outbreaks and might be expected in the current novel coronavirus pandemic. The attenuation of AMP/defensin production by lung epithelial cells may play a key role in patient susceptibility to secondary bacterial pneumonia.





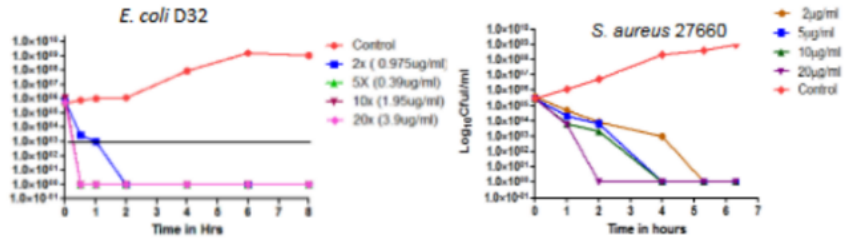
[5] Dr. Gerberding: "As we come together to fight today's Covid-19 crisis, we must also look ahead to the next one. We cannot be short-sighted, and we cannot be complacent, especially about antibiotic resistance. We must put measures in place to ensure that we have the antibiotics we need — today and in the future. The time to act is now."

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.

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

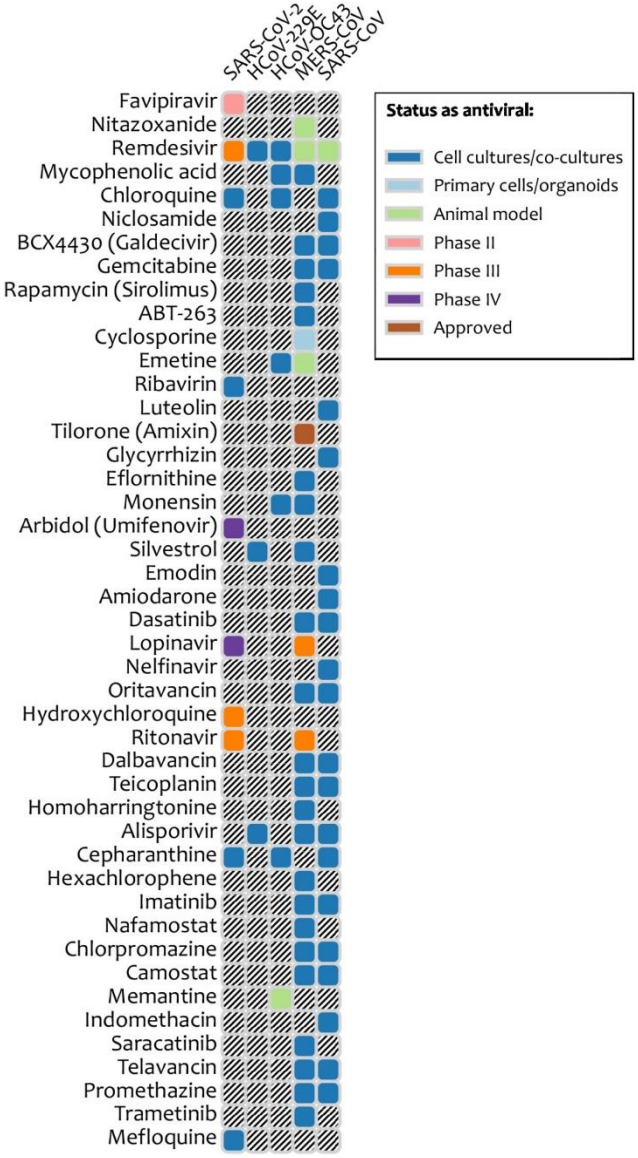
Organism Gram-negative	MIC (µg/ml) (2 - 3 isolates/organism)				
	PMX30016	PMX30063	Ceftazidime	Linezolid	Vancomycin
Citrobacter freundi	4	2 - 4	0.25 – 2	>16	>128
Citrobacter koseri	2 – 4	1 - 2	0.12 – 0.25	>16	>128
Enterobacter cloacae	2	0.5 - 4	0.25	>16	>128
Escherichia coli	2	1 - 2	0.06	>16	>128
Klebsiella 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 marcesoens	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

Table 1. Susceptibility of Clinical Isolates

Organism (# isolates)	Gram + / -	Fluoroquinolone Sensitive / Resistant	brilacidin (µg/ml)		
			MIC_{50}	MIC_{90}	MIC range
Staph. aureus (25)	+	S	0.25	0.25	0.13 – 0.5
Staph. aureus (25)	+	R	0.25	0.5	0.13 - 1
Staph. epidermidis (25)	+	S	0.13	0.25	0.03 – 0.25
Staph. epidermidis (25)	+	R	0.13	0.25	0.03 – 0.25
Strep. pneumoniae (24)	+		1	2	0.5 - 16
Strep. viridans (25)	+		4	8	1 - 32
Pseud. aeruginosa (25)	-		4	4	0.5 - 8
Haem. influenzae (25)	-		8	8	2 - 32
Serr. marcescens (25)	-		8	32	0.25 - 32

IV. Brilacidin: COVID-19 Clinical Development Pathways

Brilacidin, a leading defensin-mimetic drug candidate tested successfully in Phase 2 human trials for other clinical indications, is a promising and unique novel coronavirus (COVID-19) therapeutic candidate given Brilacidin exhibits three therapeutic properties (a 3 in 1 combination) in a single drug—antiviral, immuno/anti-inflammatory and antimicrobial.



Drug

Evaluated as an anti-inflammatory antibiotic, the Company completed a Phase 2b trial of Brilacidin, delivered as a single intravenous dose, where the drug compared favorably to a 7-day dosing regimen of daptomycin (Cubicin™).

Brilacidin could be developed, most immediately based on existing safety and efficacy data, as an intravenous drug (direct treatment), whether administered as a monotherapy or in combination, potentially leveraging synergies with other drugs or antivirals, as is being done (e.g., Hydroxychloroquine and azithromycin; antivirals and anti-inflammatories[6], e.g., remdesivir and sarilumab).

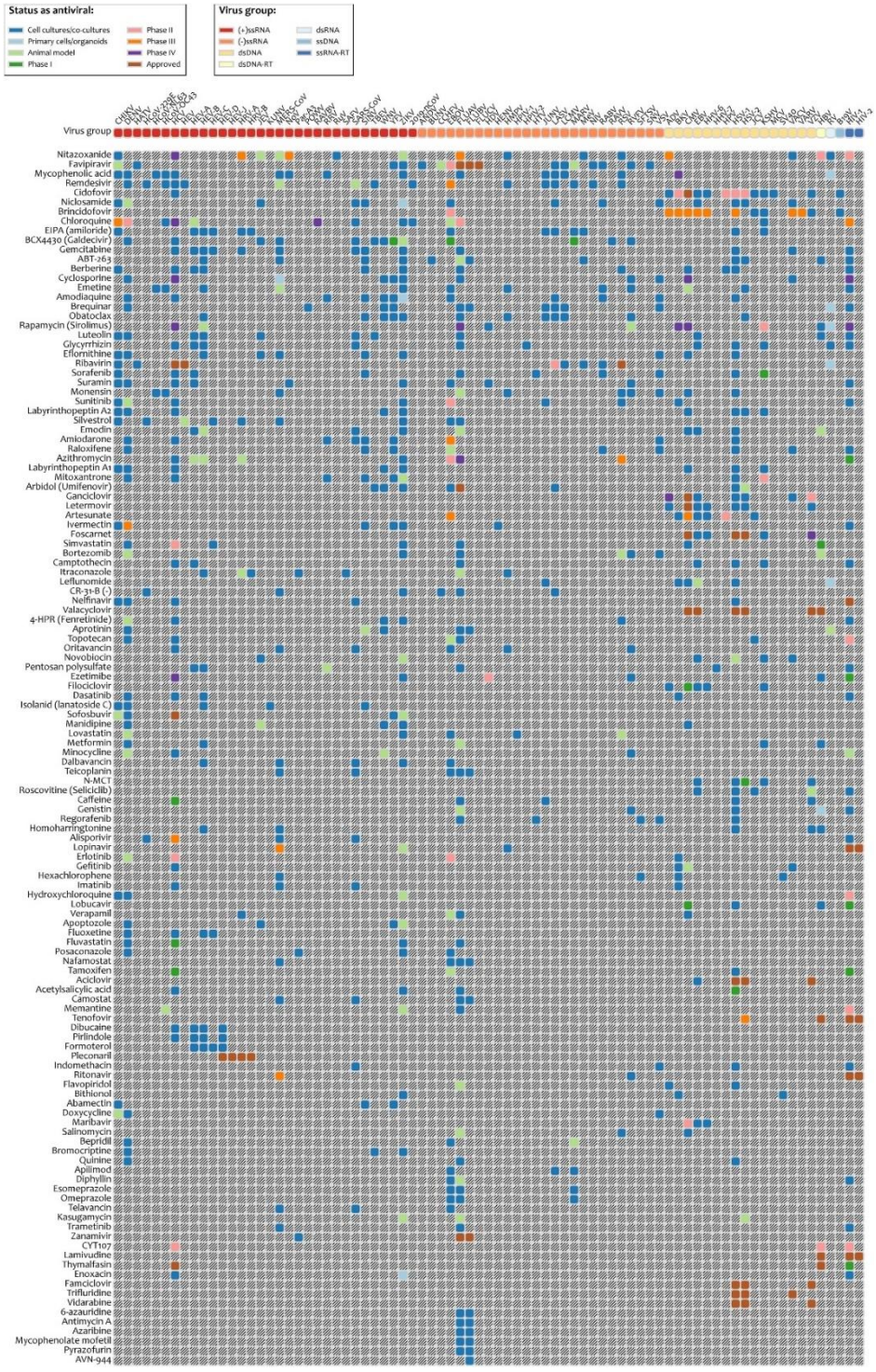
Importantly, Brilacidin is one of the few drugs currently in clinical development and among drugs already approved that has shown direct antiviral properties against SARS-CoV-2, the novel coronavirus responsible for COVID-19, in a cell-based assay, with additional research planned by academic collaborators.

Drugs that are even modestly active[7] against the novel coronavirus, particularly ones with safe therapeutic profiles having been evaluated earlier in rigorous placebo-controlled clinical trials, might not only save the lives of severely ill patients, but might also be given prophylactically to protect health care workers and others at risk of infection. Such treatments may also reduce the time patients spend in ICUs, freeing up hospital beds.

[6] See: Zhang W, et al. "The Use of Anti-inflammatory Drugs in the Treatment of People with Severe Coronavirus Disease 2019 (COVID-19): The Experience of Clinical Immunologists from China." *Clin Immunol*. 2020 Mar 25;214:108393. doi: 10.1016/j.clim.2020.108393.

[7] See: *The Brussels Times* (March 4, 2020), Gates Foundation Commissions Large Coronavirus Study from KU Leven: "But a little bit is good enough," Prof Neyts said. "Or better still, a couple of substances that each slow it down a little, that we can combine to help seriously ill patients to hopefully get better."

Additional drug delivery development work (e.g., intranasal/nebulized dosing, the use of nanotechnologies[8]) may further complement Brilacidin's anti-COVID-19 therapeutic potential.



Source

[8] See: Teixeira M, et al. "Nanomedicines for the Delivery of Antimicrobial Peptides (AMPs)" (pdf). *Nanomaterials* 2020, 10(3), 560; https://doi.org/10.3390/nano10030560; see Wnorowska U, et al. "Nanoantibiotics Containing Membrane-Active Human Cathelicidin LL-37 or Synthetic Ceragenins Attached to the Surface of Magnetic Nanoparticles as Novel and innovative Therapeutic Tools: Current Status and Potential Future Applications." (pdf) *J Nanobiotechnology*. 2020; 18: 3. Published online 2020 Jan 2.

Vaccine

Vaccines containing defensins as adjuvants have been shown, in vivo and in vitro, to activate the primary innate antiviral immune response and mediate other immunomodulatory activities against a number of viruses, including coronaviruses. A vaccine based on Brilacidin, a defensin-mimetic, is another potential clinical development pathway, though developing Brilacidin as a vaccine would involve a longer process (12-18 months) than advancing it as a COVID-19 drug candidate. Legislative and regulatory authorities, however, have indicated (pdf) a willingness to expedite vaccine development, including eliminating the need for animal studies, assigning promising vaccines Breakthrough Therapy status and making COVID-19 vaccines eligible for Priority Review Vouchers, etc. Information below and posted to the Company website includes links to literature detailing the potential of defensins developed as antiviral vaccines.

BIOMOLECULES & THERAPEUTICS

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

Towards the Application of Human Defensins as Antivirals

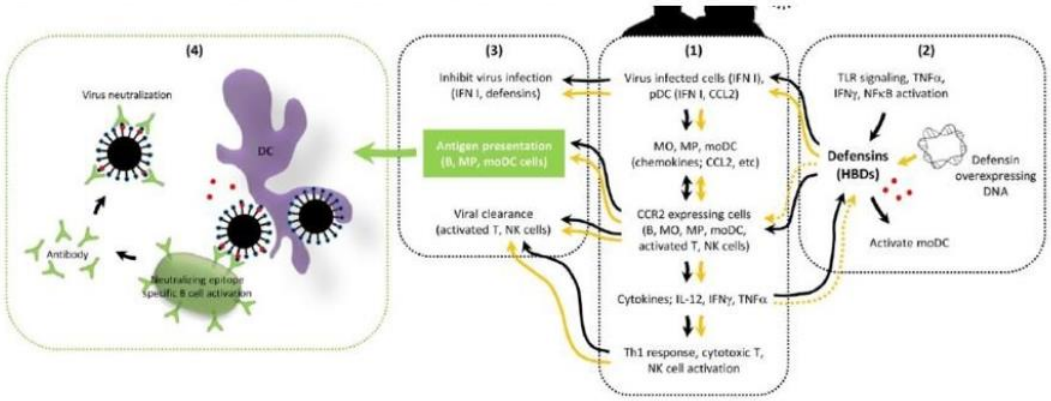
Mee Sook Park†, Jin Il Kim†, Ilseob Lee†, Sehee Park†, Joon-Yong Bae and Man-Seong Park*

Department of Microbiology, Institute for Viral Diseases, College of Medicine, Korea University, Seoul 02841, Republic of Korea

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



Source: 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.

Kim et al. Virology Journal (2018) 15:124
https://doi.org/10.1186/s12985-018-1035-2

Virology Journal

RESEARCH Open Access

Human β-defensin 2 plays a regulatory role in innate antiviral immunity and is capable of potentiating the induction of antigen-specific immunity

Ju Kim[1], Ye Lin Yang[2], Sun-Hee Jang[1] and Yong-Suk Jang[1,2*]

Abstract

Background: Antimicrobial peptides (AMPs) are primarily known for their innate immune defense against invading microorganisms, including viruses. In addition, recent research has suggested their modulatory activity in immune induction. Given that most subunit vaccines require an adjuvant to achieve effective immune induction through the activation of innate immunity, AMPs are plausible candidate molecules for stimulating not only innate immune but also adaptive immune responses.

Results: In this study, we investigated the ability of human β-defensin (HBD) 2 to promote antiviral immunity in vitro and in vivo using a receptor-binding domain (RBD) of Middle East respiratory syndrome-coronavirus (MERS-CoV) spike protein (S RBD) as a model antigen (Ag). When HBD 2-conjugated S RBD was used to treat THP-1 human monocytic cells, the expression levels of antiviral (IFN-β, IFN-γ, MxA, PKR, and RNaseL) and primary immune-inducing (NOD2, TNF-α, IL-1β, and IL-6) molecules were enhanced compared to those expressed after treatment with S RBD only. The expression of chemokines capable of recruiting leukocytes, including monocytes/macrophages, natural killer cells, granulocytes, T cells, and dendritic cells, was also increased following HBD 2-conjugated S RBD treatment. More important, immunization of mice with HBD 2-conjugated S RBD enhanced the immunogenicity of the S RBD and elicited a higher S RBD-specific neutralizing antibody response than S RBD alone.

Conclusions: We conclude that HBD 2 activates the primary antiviral innate immune response and may also mediate the induction of an effective adaptive immune response against a conjugated Ag.

Keywords: Adjuvant, Antigen, Antibody, Human β-defensin, MERS-CoV

Background

In general, vaccination materials consist of a specific antigen (Ag) and an adjuvant capable of potentiating the immunogenicity of the Ag to achieve efficient Ag-specific adaptive immunity [1]. Vaccines made up of live attenuated and/or killed whole pathogens usually contain endogenous adjuvants, such as bacterial cell wall components, genomic nucleic acids, and various pathogen-derived materials, that act as pathogen-associated molecular patterns and are sufficient to induce Ag-specific adaptive immunity by potentiating immunogenicity through the activation of innate immunity [2, 3]. However, subunit vaccines that utilize recombinant and/or purified Ags usually lack these endogenous innate immune stimulators. Consequently, the addition of exogenous materials with adjuvant activity is required to mimic natural infection to draw effective pathogenic Ag-specific adaptive immunity [4].

The innate immune response includes the production of interferons (IFNs), complements, and antimicrobial peptides (AMPs) and is crucial for controlling infectious diseases and inducing adaptive immunity [5]. AMPs have been proposed as multifunctional peptides that participate

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[2]Department of Bioactive Material Sciences and Institute of Bioactive Materials, Chonbuk National University, Jeonju 54896, Korea

Source: Kim J, et al. "Human β-defensin 2 Plays a Regulatory Role in Innate Antiviral Immunity and Is Capable of Potentiating the Induction of Antigen-Specific Immunity." *Virol J.* 2018; 15: 124. Published online 2018 Aug 8. doi: 10.1186/s12985-018-1035-2

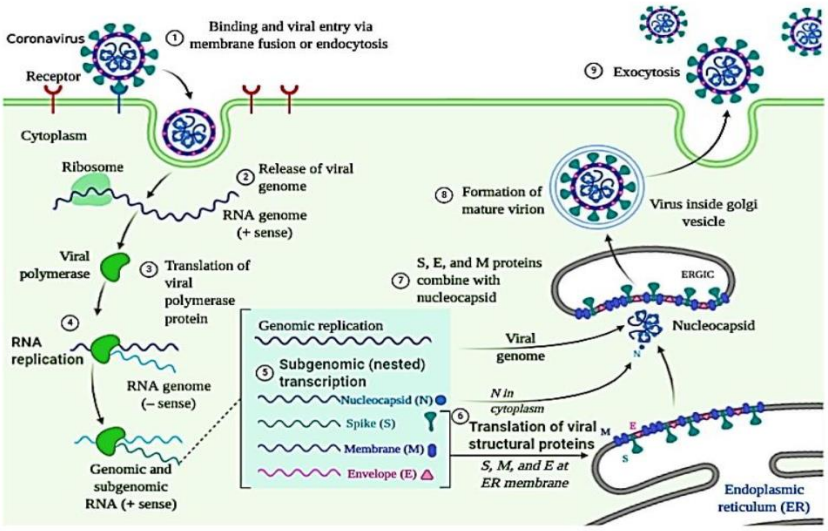

Figure-3: Figure represents the mechanisms of Coronavirus infection in host cell binding and viral entry through membrane fusion or endocytosis.

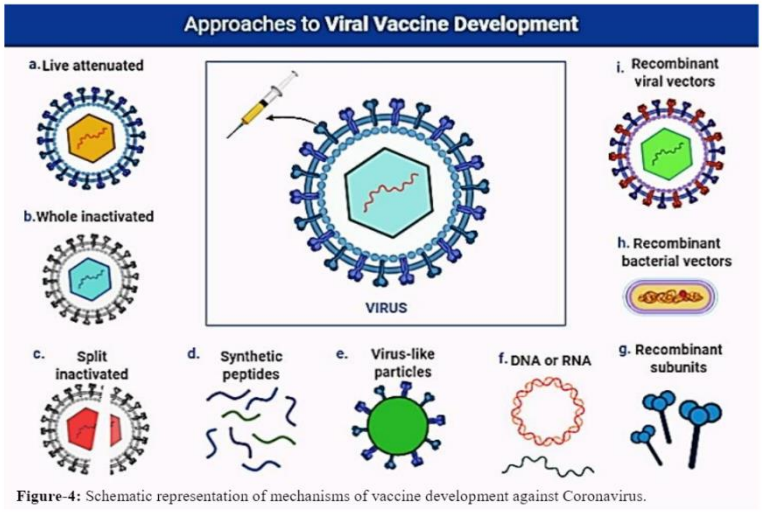

Figure-4: Schematic representation of mechanisms of vaccine development against Coronavirus.

Table-1: Summary of the development of antiviral agents and vaccine development against Coronavirus (CoV).

S. No	Drug	Status	References
1.	Favilavir	Phase-III	[53]
2.	Altimmune's intranasal vaccine	stage I clinical trial	
3.	INO-4800	Pre-clinical testing	[54]
4.	NP-120 (Ifenprodil)		
5.	APN01	Phase-I pilot trial	[55]
6.	mRNA-1273	Phase-I clinical trial	[57]
7.	Avian CoV infectious Bronchitis virus vaccine	Pre- clinical trials	[54]
8.	Brilacidin	Pre-clinical stage	
9.	Clover – recombinant subunit vaccine	Pre-clinical stage	[58]
10.	Vaxart's CoV vaccine	Pre-clinical stage	
11.	CytoDyn- leronlimab	Phase-II clinical trials	[55]
12.	Linear DNA vaccine – Takis Biotech	Pre-clinical stage	
13.	Remdesivir (GS-5734)	Phase-III clinical trials	NCT04254664
14.	Chloroquine or hydroxychloroquine	clinical trial	NCT04261517
15.	Camrelizumab and thymosin	Phase II trials	NCT04268537
16.	Azvudine	Phase I	ChiCTR2000029853

Source: Kumar D. "Understanding the Molecular Mechanism(s) of SARS-CoV2 Infection and Propagation in Human to Discover Potential Preventive and Therapeutic Approach." *OSF Preprints*. April 14, 2020.

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.

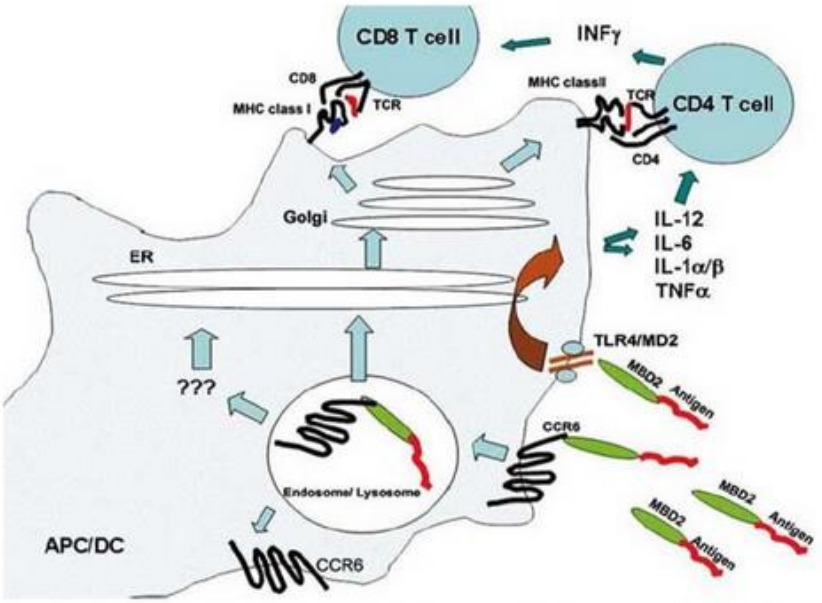


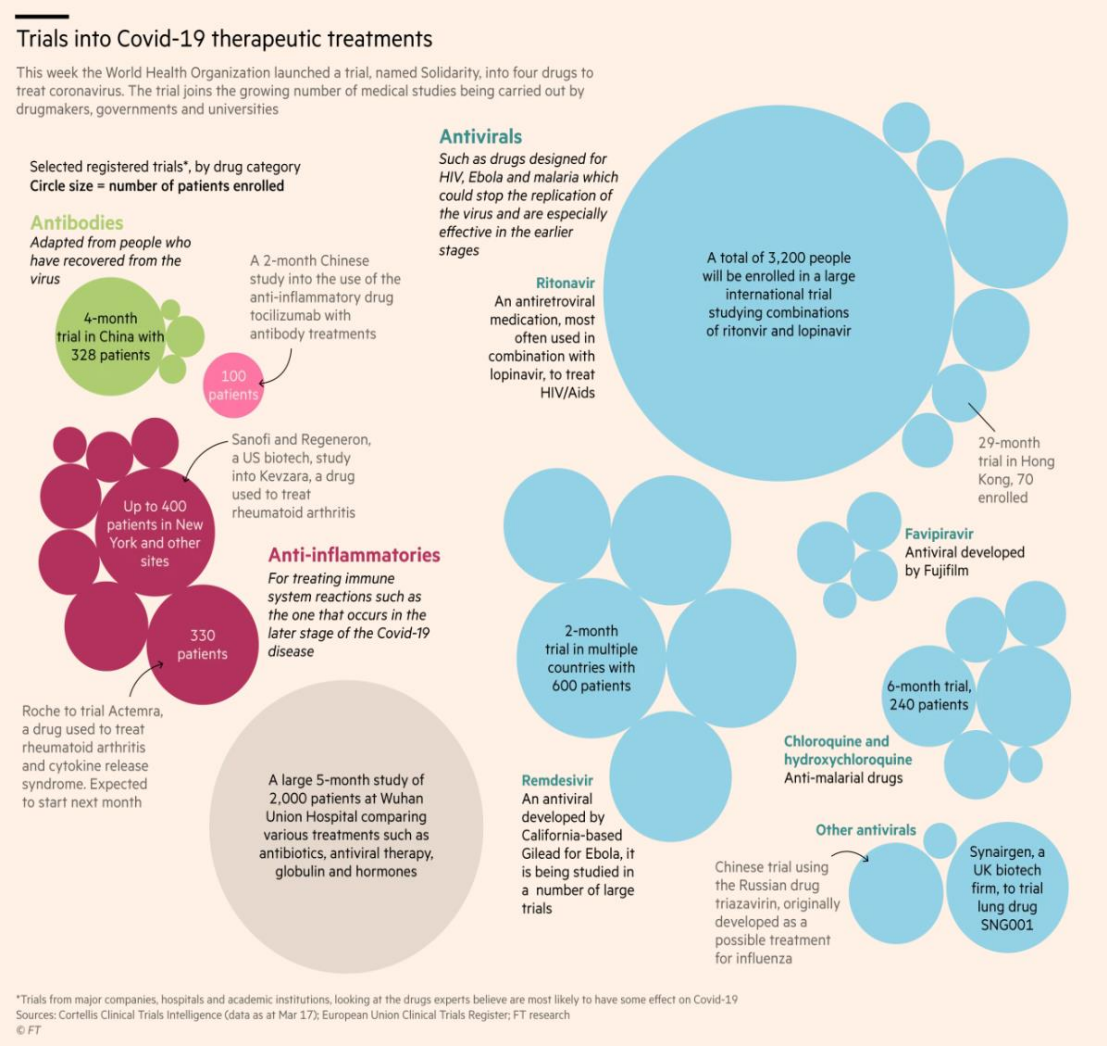
Fig. (1). Defensins as carrier for vaccines to target APCs. Murine β-defensin 2 –fused antigens target to APCs via the CCR6 chemokine receptor CCR6, which is internalized to deliver the complex to early/late endosomal compartments. The internalized defensin-antigen is processed and presented to both 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: Biragyn A. "Defensins—Non-Antibiotic Use for Vaccine Development." *Current Protein and Peptide Science*, Volume 6, Number 1, 2005, pp. 53-60(8).

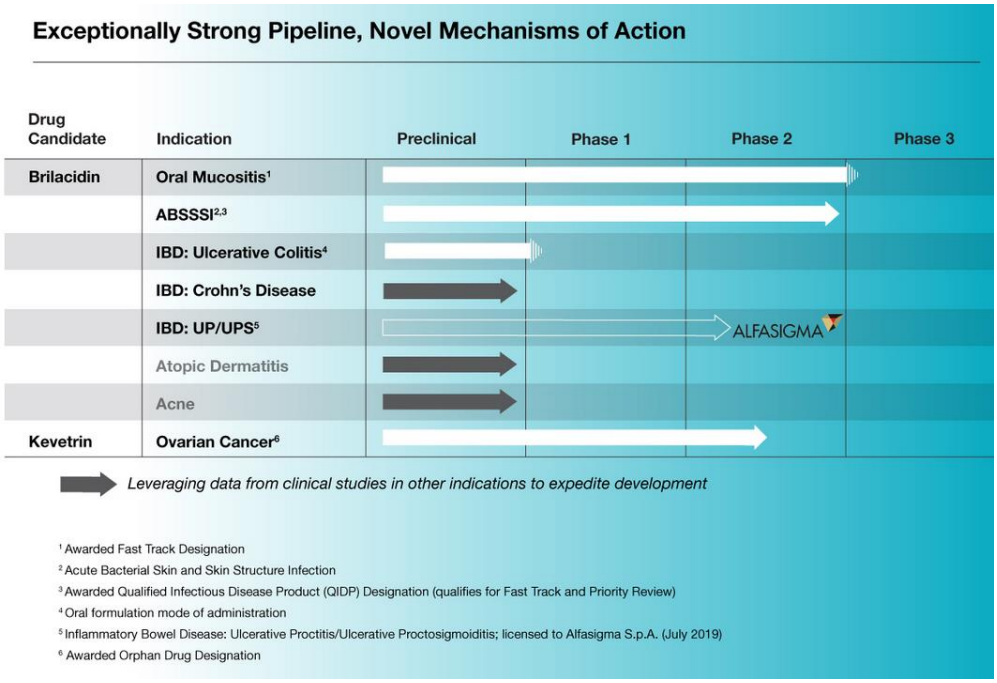
Next Steps

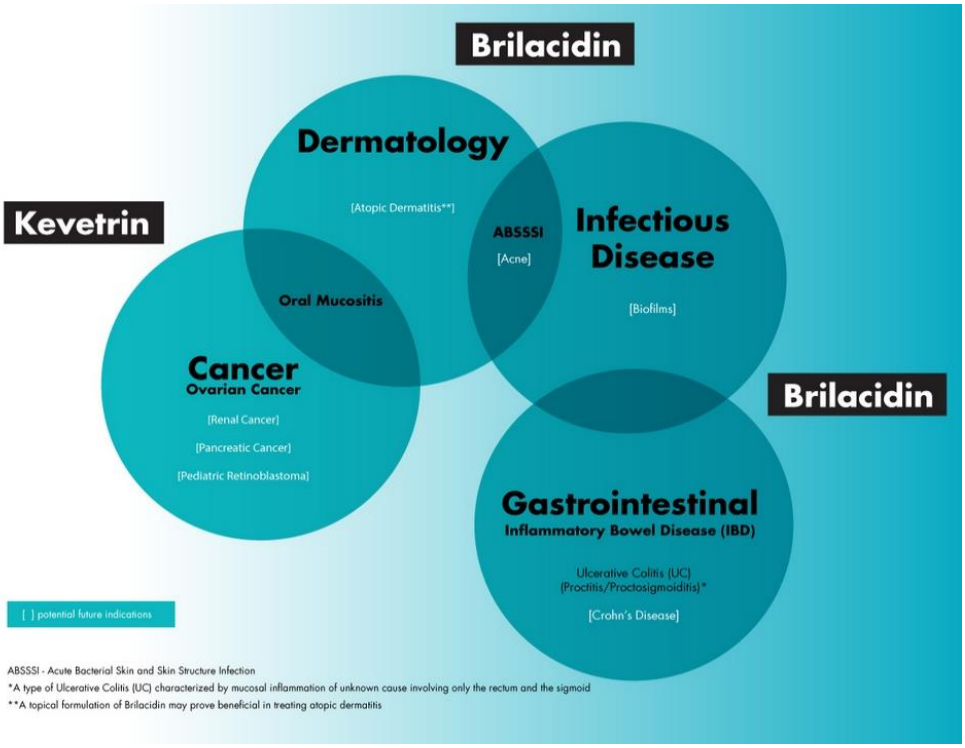
The Company is engaged in discussions within government, the pharmaceutical industry, and among health care provider networks and hospitals both in the United States and Europe, toward rapidly advancing Brilacidin testing into human trials to evaluate its potential as a novel coronavirus (COVID-19) therapeutic. Their interest in Brilacidin as a treatment for COVID-19 and associated complications is based on the drug's promising *antiviral* activity against SARS-CoV-2, as supported in preliminary testing conducted in a monkey epithelial cell line (with testing planned to continue), and the drug's established *anti-inflammatory* and *antimicrobial* properties—a 3-in-1 treatment combination. In advance of potential clinical testing, the Company is investigating procurement of appropriate drug supply (i.e., manufacture of intravenous drug product), and preparing for engagement with regulatory authorities. While there can be no assurance that a Brilacidin for COVID-19 clinical trial will commence, though that is the goal of the Company, a recent announcement by the National Institutes of Health (NIH) to launch a public-private partnership to speed COVID-19 therapeutics is a hopeful sign—that promising drugs, such as Brilacidin, might be rapidly developed to help address the COVID-19 pandemic.



Source

V. Brilacidin: Phase 2 Clinical Trial Data in Other Indications

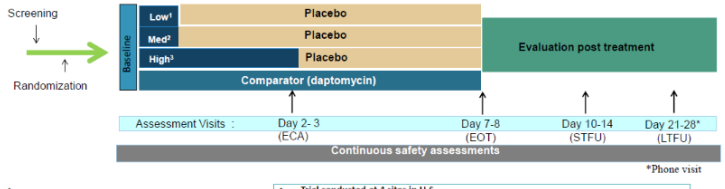




Brilacidin for Acute Bacterial Skin and Skin Structure Infection (ABSSSI)

FDA Qualified Infectious Disease Product, QIDP; Phase 2b (NCT02052388), *intravenous delivery*

Study Design



¹**Low** (0.6mg/kg single dose)
²**Med** (0.8mg/kg single dose)
³**High** (0.6 mg/kg D1; 0.3 mg/kg D2 & D3)*

**Highest total dose of 1.2 mg/kg is less than lowest total dose of 1.6 mg/kg in phase 2a study*

- **Trial conducted at 4 sites in U.S.**
- **Dosing: IV infusion 1x/day for 7 days**
  - 1or 3 days on BRI + 4 or 6 days on placebo; or 7 days on daptomycin
- **215 patients, 4 arms, ~50 patients per arm**
- **ABSSSI definition (FDA Guidance, Oct 2013)**
  - ≥ 75 sq. cm² (redness, edema, and/or induration)
  - Wound, major cutaneous abscess, cellulitis/erysipelas
- **Early Clinical Response (48-72 hours)—FDA endpoint**
- **Clinical Response (Day 7-8; Day 10-14)—EMA endpoint**

Definition	Number (%) of Subjects [a]				
	Brilacidin (mg/kg IV)				
	0.6 1 day	0.8 1 day	0.6/0.3 3 days	Daptomycin 7 days	Overall
Intent-to-Treat (ITT)	54	53	54	54	215
All Treated/Safety	53	53	53	50	209
Microbiological ITT (MITT)	31 (58.5)	35 (66.0)	29 (54.7)	38 (76.0)	133 (63.6)
Clinical Evaluable – EOT (CE-EOT)	48 (90.6)	46 (86.8)	49 (92.5)	47 (94.0)	190 (90.9)
Clinical Evaluable – STFU (CE-STFU)	49 (92.5)	42 (79.2)	45 (84.9)	46 (92.0)	182 (87.1)
Microbiological Evaluable – EOT (ME-EOT)	29 (54.7)	28 (52.8)	26 (49.1)	35 (70.0)	118 (56.5)
Microbiological Evaluable – STFU (ME-STFU)	29 (54.7)	24 (45.3)	23 (43.4)	34 (68.0)	110 (52.6)

Source: Section 14, Table 14.1.2.1.
(a) Percentages are based on the number of subjects who received at least 1 dose of study treatment in each treatment group.

FDA Early Endpoint & Safety
EMA Endpoints at EOT and STFU

- ATS Population = All subjects who received any amount of study drug
- mITT Population = All ATS subjects with ABSSSI pathogen isolated at baseline

Efficacy/Safety

Study Timepoint		0.6 mg/kg IV x 1 day	0.8 mg/kg IV x 1 day	Brilacidin x 3 days	Daptomycin
EOT	N assessed	30	31	29	38
(D7-8)	Clinical Response (%)	29 (96.7)	26 (83.9)	26 (89.7)	35 (92.1)
	95% C.I.	(90.2, 100)	(70.9, 96.8)	(78.6, 100)	(83.5, 100)
	Non-clinical Response*	1	5	3	3
STFU	N assessed	30	29	25	36
(D10-14)	Clinical Response (%)	29 (96.7)	24 (82.8)	24 (96.0)	34 (94.4)
	95% C.I.	(90.2, 100)	(69.0, 96.5)	(88.3, 100)	(87.0, 100)
	Non-clinical Response*	1	5	1	2

mITT Population

*Includes PI response of "Clinical Failure" and "Indeterminate"

	Brilacidin: 0.6 mg/kg single dose	Brilacidin: 0.8 mg/kg single dose	Brilacidin: 3-day regimen	Daptomycin
No. of Subjects	53	53	53	50
No. of Treatment-Emergent AEs	90	114	149	61
Subjects with at least 1 TEAE, n (%)	42 (79.2)	43 (81.1)	49 (92.5)	23 (46.0)
Subjects with AE leading to study withdrawal, n (%)	3 (5.7)	1 (1.9)	2 (3.8)	1 (2.0)
Subjects with at least 1 TR AE, n (%)	35 (66.0)	37 (69.8)	47 (88.7)	17 (34.0)
-Subjects with AE of numbness or tingling (N/T), n (%)	31 (58.5)	33 (62.3)	39 (73.6)	4 (8.0)
-Excluding N/T, subjects with at least 1 TRAE, n (%)	4 (7.5)	4 (7.5)	8 (15.1)	13 (26.0)
Subjects with at least 1 SAE, n (%)	3 (5.7)	1 (1.9)	2 (3.8)	0 (0.0)
Subjects with AE leading to death, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Subjects reported with AE of hypertension or BP increased, ≥160 mmHg , n (%)	2 (3.8)	9 (17.0)	14 (26.4)	5 (10.0)

Baseline Pathogens

Baseline Pathogen	PI Clinical Assessment at Day 7/8: EOT				PI Clinical Assessment at Day 10-14: STFU			
	Brilacidin				Brilacidin			
	0.6 1 day	0.8 1 day	0.6/0.3 3 days	Daptomycin 7 days	0.6 1 day	0.8 1 day	0.6/0.3 3 days	Daptomycin 7 days
Staphylococcus aureus								
MSSA only	16/17 (94.1)	15/18 (83.3)	12/13 (92.3)	11/13 (84.6)	16/17 (94.1)	14/17 (82.4)	12/12 (100.0)	11/12 (91.7)
+ *S. lugdunensis*	1/1 (100.0)		1/1 (100.0)		1/1 (100.0)		1/1 (100.0)	
+ *S. anginonsus-milleri*		1/1 (100.0)	1/1 (100.0)			1/1 (100.0)	1/1 (100.0)	
+ *S. pyogenes*				2/2 (100.0)				2/2 (100.0)
MRSA only	9/9 (100.0)	7/8 (87.5)	10/11 (90.9)	12/13 (92.3)	9/9 (100.0)	6/7 (85.7)	8/8 (100.0)	11/12 (91.7)
+ *E. faecalis*				1/1 (100.0)				1/1 (100.0)
+ *S. agalactiae*				1/1 (100.0)				1/1 (100.0)
Streptococcus								
agalactiae				1/1 (100.0)				1/1 (100.0)
anginonsus-milleri	2/2 (100.0)	2/3 (66.7)	2/3 (66.7)	3/3 (100.0)	2/2 (100.0)	2/3 (66.7)	2/3 (66.7)	3/3 (100.0)
pyogenes	1/1 (100.0)				1/1 (100.0)			
Staphylococcus								
lugdunensis		1/1 (100.0)		1/1 (100.0)		1/1 (100.0)		1/1 (100.0)
Enterococcus								
faecalis				1/1 (100.0)				1/1 (100.0)
Group C Beta-hemolytic streptococci				2/2 (100.0)				2/2 (100.0)

Pathogen [a]	$N_{isolates}$	Brilacidin MIC (µg/ml)			
		Range		MIC_{50}	MIC_{90}
		Minimum	Maximum		
S. aureus	113	0.50	2.00	1.00	1.00
MSSA	65	1.00	2.00	1.00	1.00
MRSA	48	0.5	2.00	1.00	1.00
S. pyogenes	2	2.00	2.00	-	-
S. agalactiae	2	2.00	2.00	-	-
Group C Beta- haemolytic streptococci	1	2.00	2.00	-	-
S. anginonsus-milleri group	3	0.25	4.00	2.00	4.00
E. faecalis	3	4.00	8.00	4.00	8.00
S. lugdunensis	4	1.00	1.00	1.00	1.00

(a) Based on central laboratory results.

Source (ECCMID 2015)

Brilacidin for Inflammatory Bowel Disease (IBD)

Phase 2 Proof-of-Concept in Ulcerative Proctitis/Ulcerative Proctosigmoiditis (UP/UPS), *enema formulation*; currently being developed as an *oral tablet* (NCT04240223) in Ulcerative Colitis (UC), Phase 2 planning underway

Brilacidin for IBD: Phase 2 UP/UPS

Rectal Enema Formulation demonstrated Clinical remission, supported by Endoscopic improvement

Clinical Remission in majority of patients at Week 6 (Day 42)

Similar across cohorts

- **60%** (3 of 5) in Cohort A, **50 mg Brilacidin**
- **67%** (4 of 6) in Cohort B, **100 mg Brilacidin**
- **75%** (3 of 4) in Cohort C, **200 mg Brilacidin**

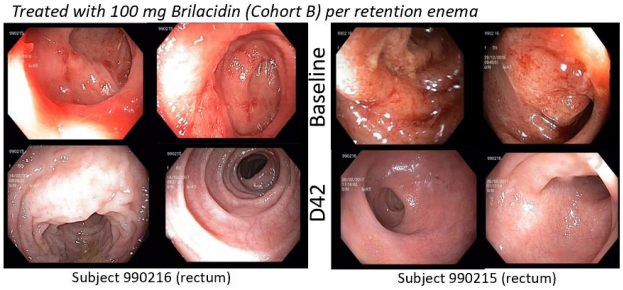
Analysis population: Includes subjects with Endoscopy, Rectal Bleeding and Stool Frequency subscores at baseline and Day 42; one patients in Cohort A and one patient in Cohort C are not included due to no Day 42 endoscopy (patients declined)

Clinical Remission defined as:

- Endoscopy subscore ≤ 1
- Rectal Bleeding subscore of 0
- Stool Frequency subscore improvement or no change from baseline

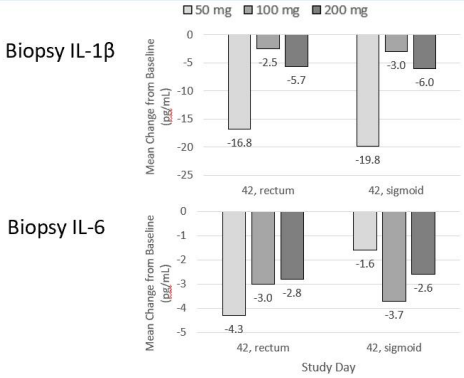
Examples Clinical Remission

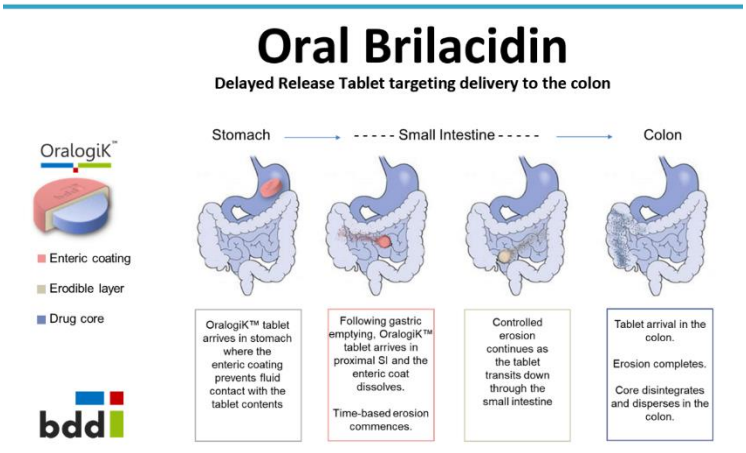
Treated with 100 mg Brilacidin (Cohort B) per retention enema

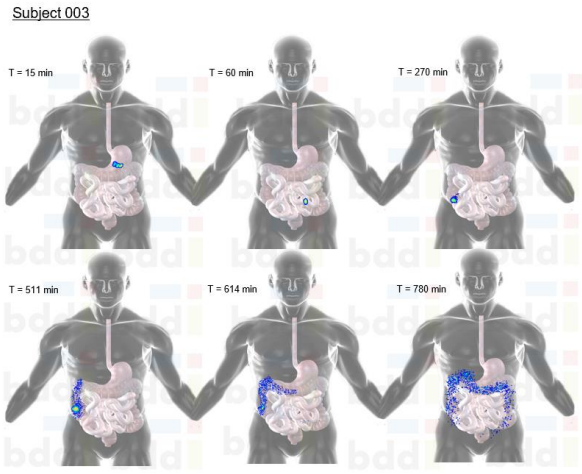


Subject 990216 (rectum) Subject 990215 (rectum)

Colonic tissue biopsies at Week 6 (D42) demonstrate reduction in inflammatory biomarkers







Source: Innovation Pharmaceuticals Inc.

Brilacidin for Oral Mucositis (OM)

FDA Fast Track; Phase 2 (NCT02324335), *oral rinse delivery*; Phase 3 planning underway

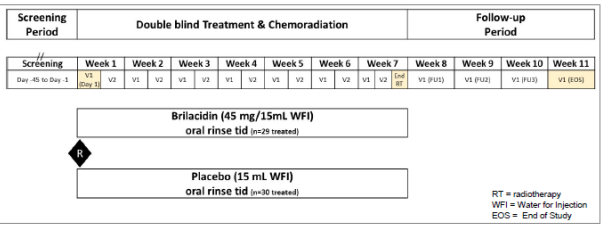
CTIX-BRI-205: Oral Mucositis Phase 2 Study

Efficacy and Safety of Brilacidin oral rinse administered tid for 7 weeks (49 days)

- Phase 2, multi-center (USA), randomized, double-blind, placebo-controlled study
- Daily treatment aimed at attenuating Oral Mucositis (OM) in subjects with Head and Neck Cancer receiving concurrent chemoradiation therapy

Design:

- 7 weeks of treatment, with two study visits per week
- 2 treatment groups:
  - Brilacidin (45 mg/15 mL WFI, tid)
  - Placebo (15 mL WFI, tid)
- Oral rinse (15 mL); "swish" for 1 min, then "spit" out
- 3 x daily oral rinse (tid), approximately 6 hours apart



Patients:

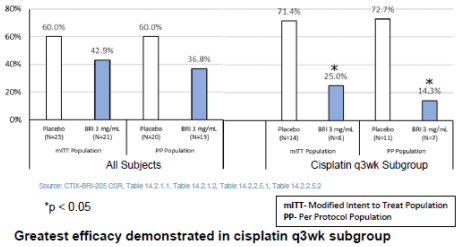
- Recently diagnosed (within previous 3 months), pathologically confirmed, non-metastatic squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, or supraglottic larynx
- Radiation Therapy: at least 2 oral sites to receive single daily fractions of 2.0-2.2 Gy, with a cumulative radiation dose ≥ 55 Gy and ≤ 72 Gy
- Chemotherapy: cisplatin weekly (30-40 mg/m^2) or approximately every 21 days (80-100 mg/m^2)

CTIX-BRI-205: Oral Mucositis Phase 2 Study

Primary Efficacy Endpoint met

Brilacidin oral rinse met primary endpoint of **reduced incidence of severe OM (WHO Grade ≥ 3)** experienced by subjects during chemoradiation therapy

Incidence of SOM (WHO Grade ≥ 3)



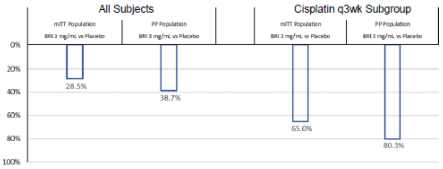
Source: CTIX-BRI-205 CSR, Table 14.2.1.1, Table 14.2.1.2, Table 14.2.2.5.1, Table 14.2.2.5.2

*p < 0.05

mITT- Modified Intent to Treat Population
PP- Per Protocol Population

- **Greatest efficacy demonstrated in cisplatin q3wk subgroup**
- **Incidence of SOM on placebo** was **60% (in this study)** [~70+% historically]

Relative Difference (Reduction) in Incidence of SOM, Brilacidin vs placebo



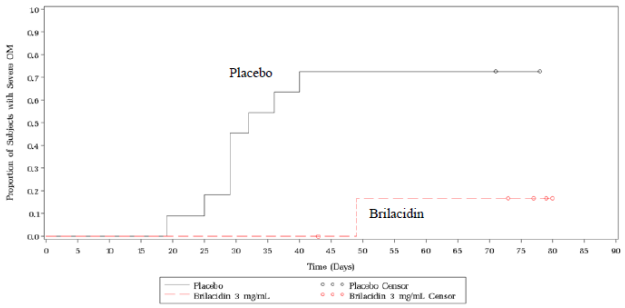
Note: Relative difference = (incidence [placebo] – incidence [Brilacidin]) / incidence [placebo]

mITT Population = All randomized subjects who received at least one dose of study drug and a cumulative radiation dose of at least 55 Gy (and no more than 72 Gy)

CTIX-BRI-205: Phase 2 Oral Mucositis Trial

Positive Results: Delayed Time to Onset *of Severe Oral Mucositis marked in 21-day Cisplatin subgroup*

Kaplan-Meier Curves for Time to Onset, in Days, of Severe OM, 21-day Cisplatin Schedule (PP Population)



Note the period from approximately **19-49 days** during which SOM incidence rises strikingly in Placebo while not in the Brilacidin group

Source: Innovation Pharmaceuticals

Brilacidin Platform Potential

Gateway Concept Given Wide Range of HDP-M Therapeutic Activity

Innate Immunity

Pathogen Defense

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

VI. AMPs/Defensins (Mimetics): Antiviral Properties

Antiviral properties of natural AMPs/defensins and their synthetic mimics are actively being studied by scientists 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:

- 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.*** *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." [emphasis added]*

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]

- Brice DC, and Diamond, G. "Antiviral Activities of Human Host Defense Peptides." *Curr Med Chem.* 2019 Aug 5. doi: 10.2174/0929867326666190805151654. [Epub ahead of print]

  "Due to their common structural features, including an amphipathic structure and cationic charge, they [HDPs] have been widely shown to interact with and disrupt microbial membranes. Thus, it is not surprising that human HDPs have activity against enveloped viruses as well as bacteria and fungi. However, these peptides also exhibit activity against a wide range of non- enveloped viruses as well, acting at a number of different steps in viral infection. […] ***The broad spectrum of antiviral activity of these peptides****, both in vitro and in vivo suggest that* ***they play an important role in the innate antiviral defense against viral infections****. Furthermore, the literature suggests that* ***they may be developed into antiviral therapeutic agents****." [emphasis added]*

Table 1. Known antiviral activities of human defensins against enveloped DNA viruses [21].

Virus\Defensin	HNP1	HNP2	HNP3	HNP4	HD5	HD6	HBD1	HBD2	HBD3	HBD4
Herpesviruses	-	-	-	-	-	-	-	-	-	-
CMV	U	U	U	*	*	*	*	*	*	*
HSV-1	U	U	U	*	U	*	U	*	U	*
HSV-2	Binds gB to prevent attachment, inhibits VP16 transport and viral gene expression post-infection	Binds gB to prevent attachment, inhibits VP16 transport and viral gene expression post-infection	Binds gB to prevent attachment, inhibits VP16 transport and viral gene expression post-infection	Binds to heparan sulfate to prevent attachment	Binds gB to prevent attachment, inhibits viral gene expression post-infection	Binds to heparan sulfate to prevent attachment	NE	NE	Binds to gB and heparan sulfate to prevent attachment	*
VZV	*	*	*	*	*	*	*	U	*	*
Vaccinia	NE	*	*	*	*	*	NE	NE	U	*

NE: Tested HDP had no effect, U: Antiviral mechanism is undetermined, *: Yet to be tested. Table shows most common result of HDP antiviral test.

Table 7. Direct comparisons of human HDP activity against viruses.

Virus	Direct comparison of HDP activity
HSV-1	HNP1, HNP2, and HNP3 were about equal in effectiveness
HSV-2	HNP1 and HD5 were most effective among defensins tested. HBD3 was more effective than HBD1
VZV	HBD2 and LL-37 had similar activities, but unlike with HBD2, LL-37 pre-incubation of virus and peptide before infection increased antiviral activity compared to after infection
Vaccinia	HBD2, but not HBD1 or HNP1/2 had effect. This effect was more potent than LL-37
HAdV	For HNP1 and HD5, viral species A, B1, B2, C, and E susceptible, but D and F are not. HD5 was more effective than HNP1 against HAdV5 and HAdV35. HBD1/2 and HD6 mostly ineffective. Defensins and LL-37 inhibit different species.
HPV	HD5 had highest efficacy. HBD1/2 and HD6 had no effect. HNP1-4 and LL-37 had similar efficacies
BKV	HD5 had highest efficacy. HNP1 and HBD2 had similar effect, while HBD1 had no effect
JCV	HD5 had highest efficacy. Discrepancy for activity of HNP1 and HBD1/2. No effect seen in HNP3 or HBD4
SV40	HD5 inhibited, while HNP1 and HBD1/2 did not
HCV	Mixture of HNP1-4 (about as effective as LL-37) was more effective than mixture of HBD1-5+116
Influenza A virus	HNP3, HBD1, and HBD2 did not increase IAV uptake by neutrophils, unlike HNP1/2 and HD5. HNP1/2 (with slight edge to HNP2) more effective than HBDs or LL-37. HNP1/2 work synergistically with LL-37
RSV	HBD2 had most activity while HBD1 was ineffective. LL-37 was less effective than HBD2
HIV-I	LL-37 and HNP4 are more effective than HNP1-3, most likely due to HNP4 less binding to serum. Combination of HBD2 and HBD3 was more effective than either HBD alone. Conflicting data about HBD1 activity, with HBD2/3 having more activity. HBD2 inhibits single stage infection and provides long term antiviral activity, unlike HBD1.

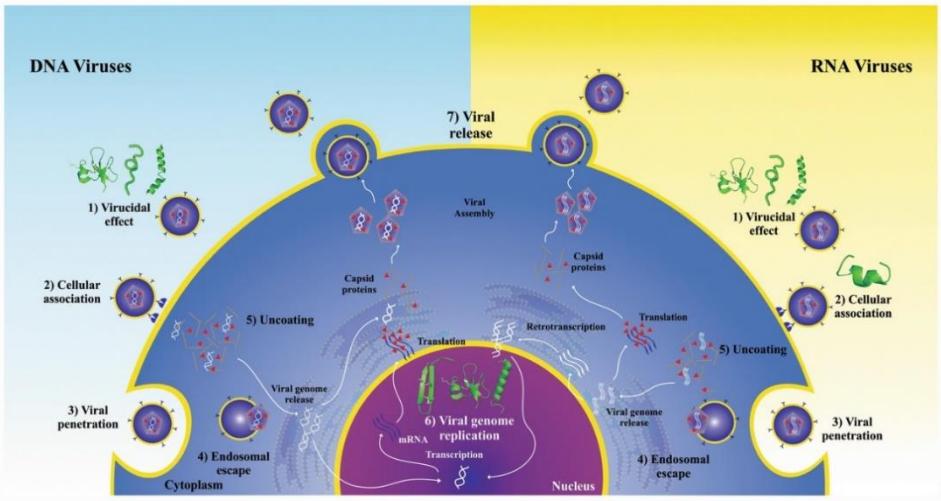
Comparisons are only between HDPs tested in the same experiment.

- Vilas Boas, LCP et al. "Antiviral Peptides as Promising Therapeutic Drugs." *Cell Mol Life Sci.* 2019 Sep;76(18):3525-3542. doi: 10.1007/s00018-019-03138-w. Epub 2019 May 17.

  "While scientific advances have led to large-scale production and widespread distribution of vaccines and antiviral drugs, **viruses still remain a major cause of human diseases today**. The ever-increasing reports of viral resistance and **the emergence and re-emergence of viral epidemics pressure the health and scientific community to constantly find novel molecules with antiviral potential**. This search involves numerous different approaches, and the use of antimicrobial peptides has presented itself as an interesting alternative. Even though the number of antimicrobial peptides with antiviral activity is still low, they already show immense potential to become pharmaceutically available antiviral drugs. Such peptides can originate from natural sources, such as those isolated from mammals and from animal venoms, or from artificial sources, when bioinformatics tools are used. **This review aims to shed some light on antimicrobial peptides with antiviral activities against human viruses and update the data about the already well-known peptides that are still undergoing studies, emphasizing the most promising ones that may become medicines for clinical use.**" […]

  Human coronaviruses are positive-sense RNA enveloped viruses that belong to the Coronaviridae family. So far, six coronaviruses (CoV) have been reported to infect humans: HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), and the Middle East respiratory syndrome coronavirus (MERS-CoV) [160]. While HCoV-229E and HCoV-OC43 are associated with upper and mild respiratory tract infections, SARS-CoV and MERS-CoV cause a variety of severe flu-like symptoms and were **responsible for recent epidemics** (in 2002/3 and 2015, respectively) [161, 162]. […]

  The peptides named 229E-HR1P and 229E-HR2P both showed inhibition of cell–cell spread, and inhibition of the pseudovirus infection, but 229E-HR2P was much more effective. Besides, in vivo assays showed that 229E-HR2P could retain its antiviral activity in both upper and lower respiratory tracts when administered intranasally. In the end, **the authors suggested that 229E-HR2P could become an antiviral drug to be used along with different antiviral molecules with a different mechanism of action, possibly exerting synergistic activity**."



Antiviral peptide inhibition sites on viral replication cycle. The antiviral peptides with a described mechanism of action were placed in their inhibition sites as follows: 1, virion inhibition; 2, adsorption; 3, viral penetration; 4, endosomal escape; 5, viral uncoating; 6, viral genome replication and 7, release of mature virions

VII. AMPs/Defensins (Mimetics): Anti-Coronavirus Potential

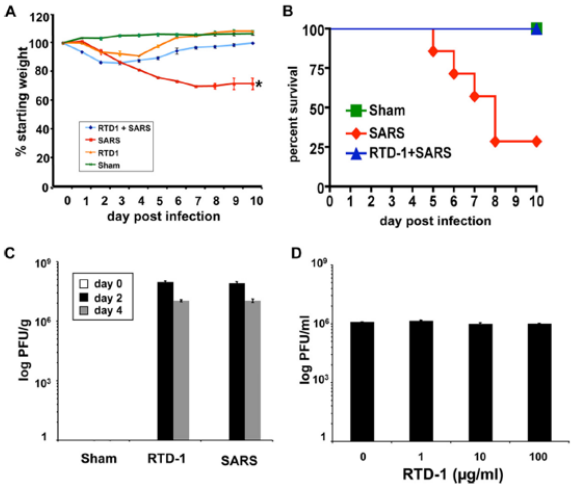
Direct Anti-Coronavirus Properties (SARS-CoV/MERS-CoV)

In the search for effective therapies to treat SARS-CoV infections, a comprehensive 2016 review article highlighted small molecule drugs and drugs that mimic peptides and proteins, like Brilacidin.

- Pillaiyar T, et al. "An Overview of Severe Acute Respiratory Syndrome–Coronavirus (SARS-CoV) 3CL Protease Inhibitors: Peptidomimetics and Small Molecule Chemotherapy." J Med Chem. 2016 Jul 28;59(14):6595-628. doi: 10.1021/acs.jmedchem.5b01461. Epub 2016 Feb 29.

Recently, intestinal lectin-like defensins were shown in cellular assays to inhibit SARS-CoV-2 binding to ACE2 and that their exogenous supplement to the lung might be therapeutic. Other research shows the AMP rhesus θ-defensin 1 (RTD-1) to have an anti-SARS-CoV effect in animal studies when administered intranasally. Note: Mice in the study were protected from lethal SARS-CoV via mechanisms that were independent of an antiviral effect, as RTD-1 was not virus neutralizing: "RTD-1 administration appeared to protect infected animals by reducing pulmonary inflammation and suppressing IL-1α, IL-1β, IL-6, IL-12, CXCL1 (KC), CCL2 (MCP-1), CCL3 (MIP-1α), and CCL5 (RANTES) 2–4 days post-infection." Further research on RTD-1 suggests the suppression of pro-inflammatory cytokines and blockade of TNF may be RTD-1's primary mechanism of action against SARS-CoV infections.

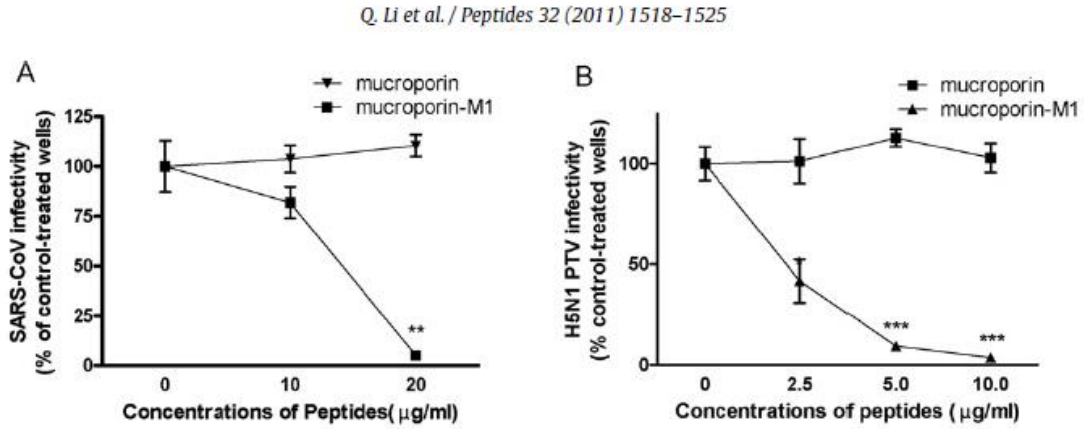
- Wohlford-Lenane CL, et al. "Rhesus Theta-Defensin Prevents Death in a Mouse Model of Severe Acute Respiratory Syndrome Coronavirus Pulmonary Disease." *J Virol*. 2009 Nov;83(21):11385-90. doi: 10.1128/JVI.01363-09. Epub 2009 Aug 26.



A February 13, 2020, Review Article—"Potential Interventions for Novel Coronavirus in China: A Systematic Review" (pdf)—refers to a peptide mimetic (Mucroporin-1) of scorpion venom, a research area of great interest among academics, that has also been shown, in pre-clinical studies, to have antiviral activity, in measles, influenza H5N1 and SARS-CoV.

- Li Q, et al. "Virucidal Activity of a Scorpion Venom Peptide Variant Mucroporin-M1 Against Measles, SARS-CoV and Influenza H5N1 Viruses." *Peptides*. 2011 Jul;32(7):1518-25. doi: 10.1016/j.peptides.2011.05.015. Epub 2011 May 19.

  "**[A]pproved or universally recommended therapies have been lacking for SARS-CoV** and influenza H5N1 infections until now, even though more and more antiviral agents against SARS-CoV and influenza H5N1 have been reported [8], [17], [24]. Therefore, the development of new antiviral agents is needed to provide more options for managing cases of diseases caused by RNA viruses in both developed and developing countries. […] **This report provides evidence that host defense peptides from scorpion venom can be modified for antiviral activity by rational design and represents a practical approach for developing broad-spectrum antiviral agents, especially against RNA viruses.**" [emphasis added]



A novel synthetic peptide (P9) derived from mouse β-defensin-4 has shown, in pre-clinical studies, broad and potent antiviral activity against respiratory viruses, including against SARS-CoV and MERS-CoV.

Two Review Articles, excerpted below, suggest AMPs/defensins can be successful therapeutic options for treating Middle East Respiratory Syndrome coronavirus (MERS-CoV), particularly those approaches that have been biocomputationally-designed (mimetics) to deliver greater efficacy (see Falanga, A et al, 2017 "Cyclic Peptides as Novel Therapeutic Microbicides: Engineering of Human Defensin Mimetics").

- Mustafa S, et al. "Current Treatment Options and the Role of Peptides as Potential Therapeutic Components for Middle East Respiratory Syndrome (MERS): A Review." *J Infect Public Health*. 2018 Jan - Feb;11(1):9-17. doi: 10.1016/j.jiph.2017.08.009. Epub 2017 Aug 31.

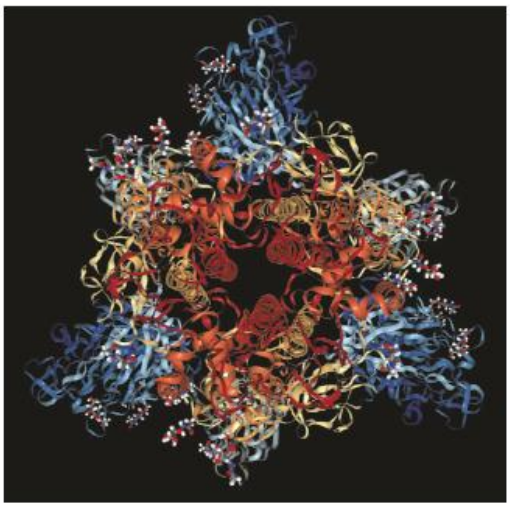
  "Currently, several therapeutic options have been employed, such as convalescent plasma (CP), intravenous immunoglobulin (IVIG), monoclonal antibodies and repurposing of existing clinically approved drugs. However, these therapeutic options have drawbacks, thus the need for an alternative approach. The requirement for effective therapeutic treatment has brought the necessity for additional MERS treatments. ***We suggest that antimicrobial peptides (AMPs) may be used as alternative therapeutic agents against MERS-CoV infection.*** *In addition, we propose the feasibility of developing effective agents by repurposing the existing and clinically approved anti-coronavirus and antiviral peptide drugs." [emphasis added]*

- Mustafa S, et al. "Peptide-Protein Interaction Studies of Antimicrobial Peptides Targeting Middle East Respiratory Syndrome Coronavirus Spike Protein: An In Silico Approach." *Advances in Bioinformatics*. Volume 2019 |Article ID 6815105 | 16 pages https://doi.org/10.1155/2019/6815105

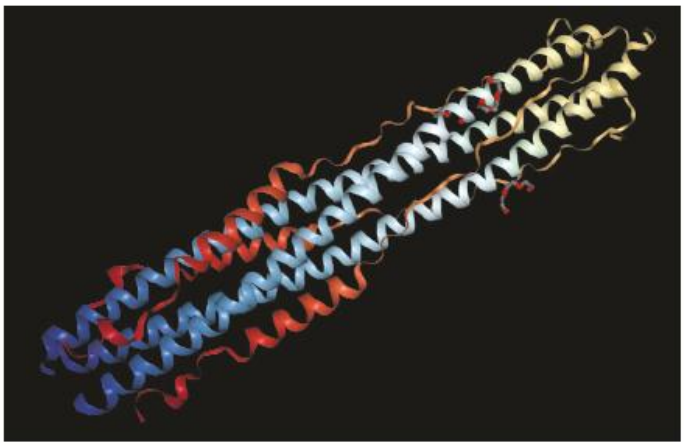
> *"It is for these reasons* ***we propose that antimicrobial peptides (AMPs) can be used as effective therapeutic agents against MERS****. Several peptides have been extensively studied and identified as* ***anti-MERS-CoV peptides*** *[9–12] and* ***anti-MERS-CoV AMPs*** *in the past few years [13]. […]*
>
> *Our computational study confirms that four AMPs were able to bind clearly to the specific binding site of S protein (5X59). From our results, it may confirm that* ***these AMPs may be suitable for inhibiting MERS-CoV virus entry into the host cell by binding and preventing fusion.*** *However, the results are preliminary and certainly need experimental confirmation using in vitro and in vivo experiments essential to validate them. Special assays studies are needed to confirm the mechanism of action. Considering all the structural aspects and binding affinity studies of the four* ***AMPs may possibly be the first choice as an anti-MERS-CoV AMPs which ould be*** **exploited to design potential inhibitors for treating MERS**."

Table 3: ClusPro ranking of docked complex based on cluster size (member), where peptides with (∗) represent the experimentally validated against MERS-CoV and are considered as positive controls.

Rank	Peptide	Length	Definition	Species	Representative	Member
1	AP00166	25	Pleurocidin	Fish	Center	134
2	AP00641	33	Pardaxin 1	Fish	Center	134
3	AP00144	23	Magainin 2	Frog	Center	117
4	AP00771	23	Magainin 1	Frog	Center	117
5	AP01644	30	Brevinin-2-RN1	Frog	Center	117
6	AP00764	24	Dermaseptin-S9	Frog	Center	110
7	AP02571	31	Cycloviolacin VY1 (cyclotides)	Plant	Center	110
8	AP00275	31	Circulin B (cyclotides)	Plant	Center	107
9	AP01022	31	Cycloviolin A (cyclotides)	Plant	Center	107
10	AP01061	31	Circulin D (cyclotides)	Plant	Center	107



(a) Prefusion stage

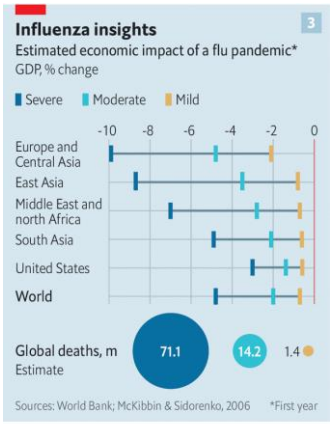


(b) Postfusion stage

VIII: The Broader Context: Characteristics of the COVID-19 Pandemic

Prevalence and Impact

The novel coronavirus (COVID-19) pandemic poses a significant life-threatening and economic risk throughout the world, with the potential eventually to infect hundreds of millions (or more) of people. Epidemiologists estimate 50 to 70 percent of the world's population will become infected. As of April 19, 2020, over 2.37 million cases have been diagnosed in at least 185 countries, resulting in almost 164,000 reported deaths, including over 742,000 cases and over 16,000 fatalities in the U.S. Presently, there are no effective approved therapies to treat COVID-19. Efforts globally are focused on aggressive containment and quarantine strategies to "flatten the curve" of the contagion so COVID-19 cases do not overwhelm a country's health care system's capacity to treat patients. Without such interventions, based on modeling performed (pdf) in 2006 by Australian researchers who studied the potential financial impact of a worldwide flu pandemic (a scenario comparable to the current COVID-19 outbreak), the monetary loss to the worldwide economy—as a measure of lost GDP—could be in the many trillions of dollars. Which this global pandemic already has surpassed.



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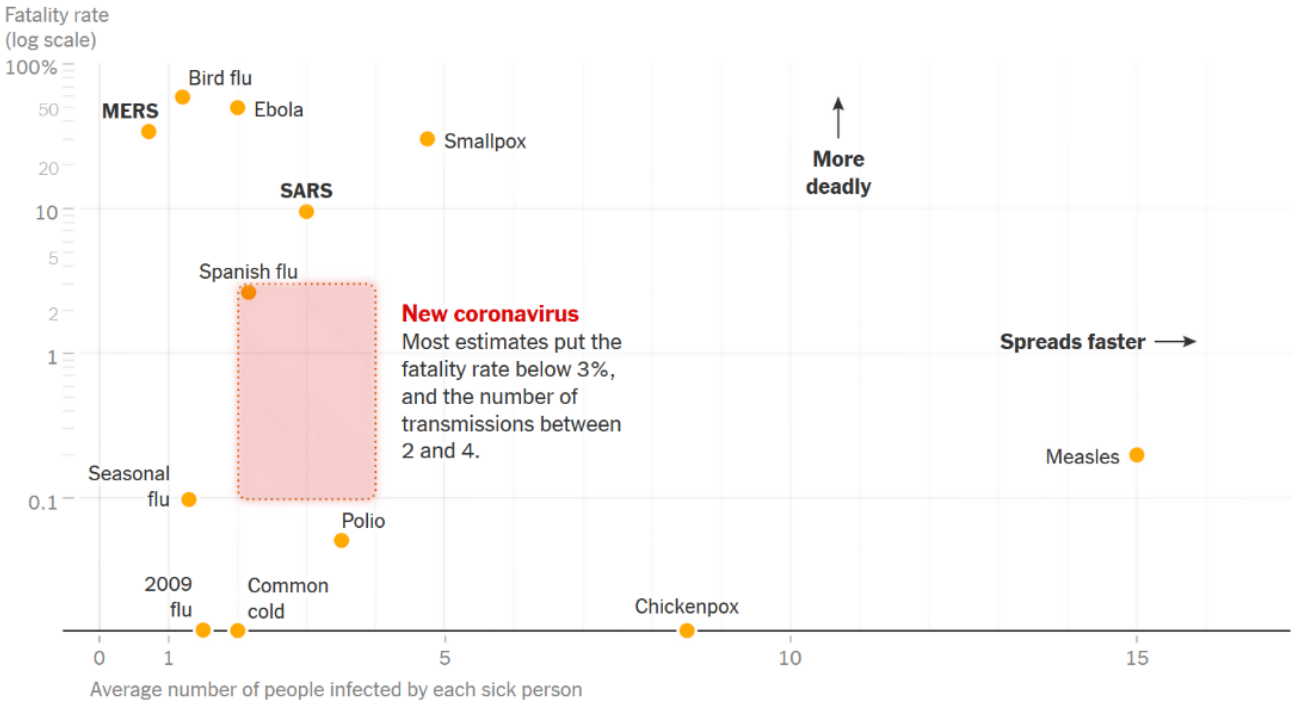

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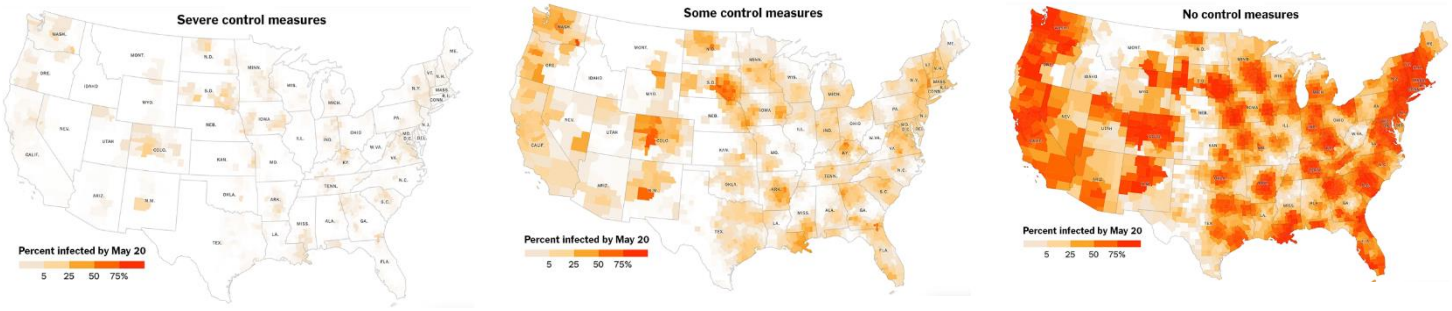
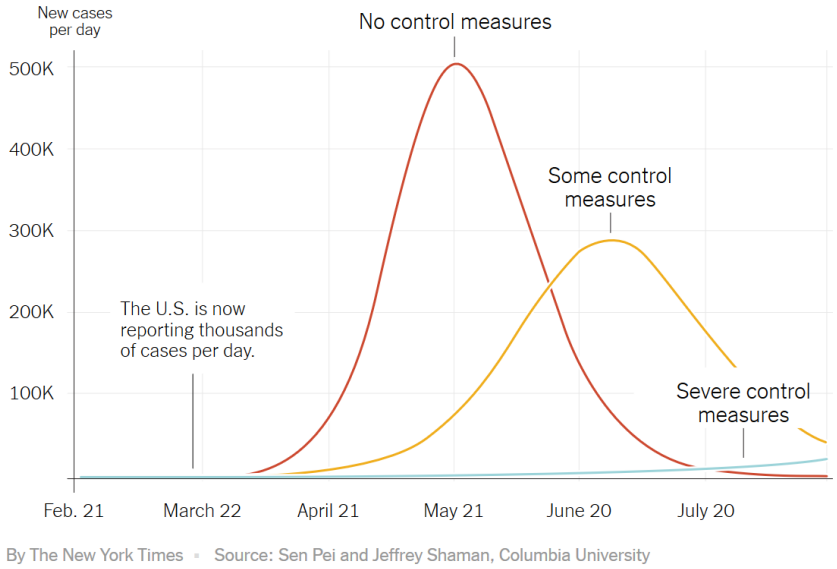


How Control Measures Could Slow the Outbreak



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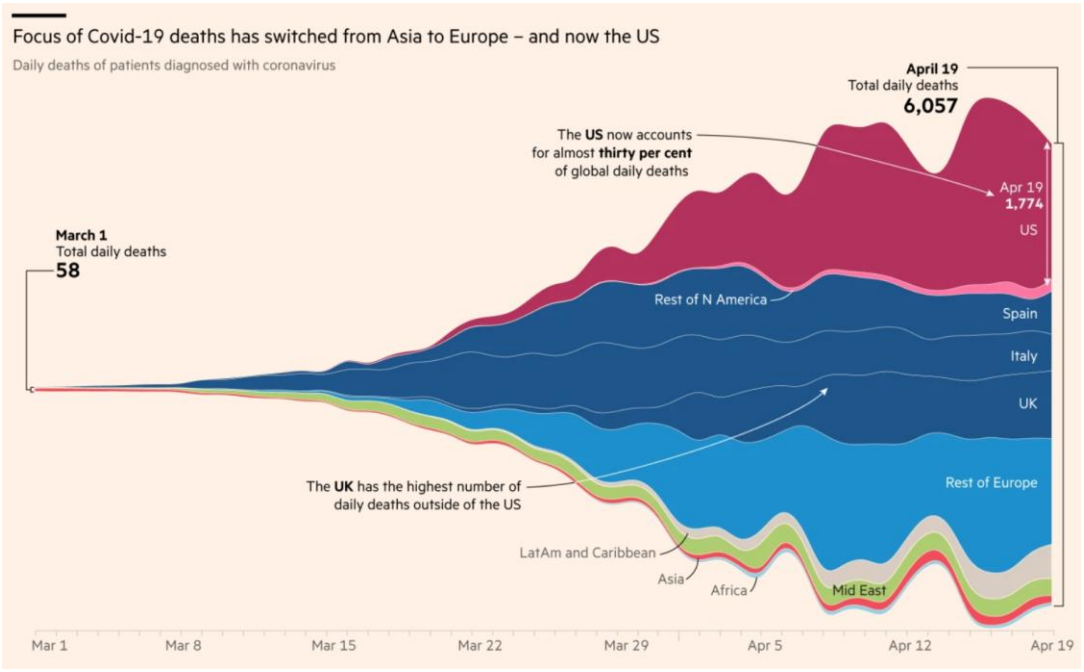
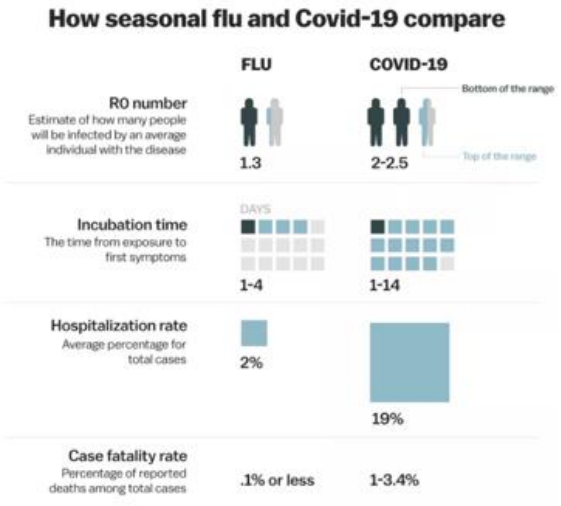


Source (data as of 4.19.2020)

Epidemiological Information

While mortality rates differ by location and by the method of calculation, there is evidence COVID-19—which has been shown to be of natural origin through genomic analyses—may be at least 10-20x more deadly than the seasonal flu (see this analysis), with an overall case mortality rate of 1.38% (1.23-1.53, 95% CrI). And it is proving to be many times more deadly among symptomatic cases and certain patient populations.[9] Children under the age of 5 may (pdf) also be more susceptible to COVID-19 than previously thought, though suffering less severely than adults.



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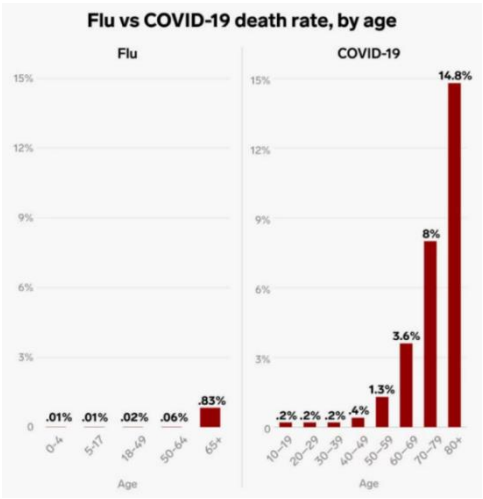


Source

Additional data: Digestive symptoms are (pdf) common and blood Type O people may be more resistant to COVID-19, along with women. Genetics is likely to play a role. The disease may at first seem to build slowly ("a slow burn"), only to intensify quickly. Serious cardiac-related complications may accompany COVID-19 disease. Warmer temperatures may help to slow the spread of the disease. Questions concerning potential reinfection have been raised by Korean health authorities. Also see "seven important things" about the coronavirus and "facts and myths" about SARS-CoV-2.

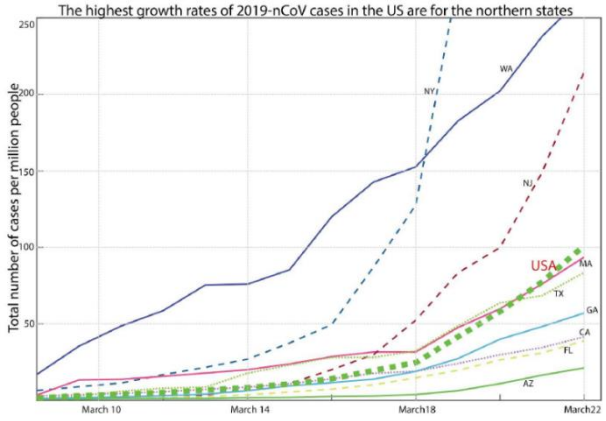


Figure 2: Growth curve of 2019-nCoV cases for different US states (until March 19,2020). The figure shows the total number of cases (normalized by the population of the state)) between March 7 and March 22, 2020.. Different states clearly follow different growth curves. The y-axis has been capped at 250 cases. NY: New York, WA: Washington, NJ: New Jersey, MA: MAssachusetts, TX: Texas, GA: Georgia, CA: California, FL: Florida and AZ: Arizona.

Source

[9] See: Baud D, et al. "Real Estimates of Morality Following COVID-19 Infections." *The Lancet*. Correspondence (March 12, 2020); see analysis by the Imperial College of London "Impact of Non-Pharmaceutical Interventions (NPIs) to Reduce COVID-19 Mortality and Healthcare Demand" (March 16, 20202) (pdf).

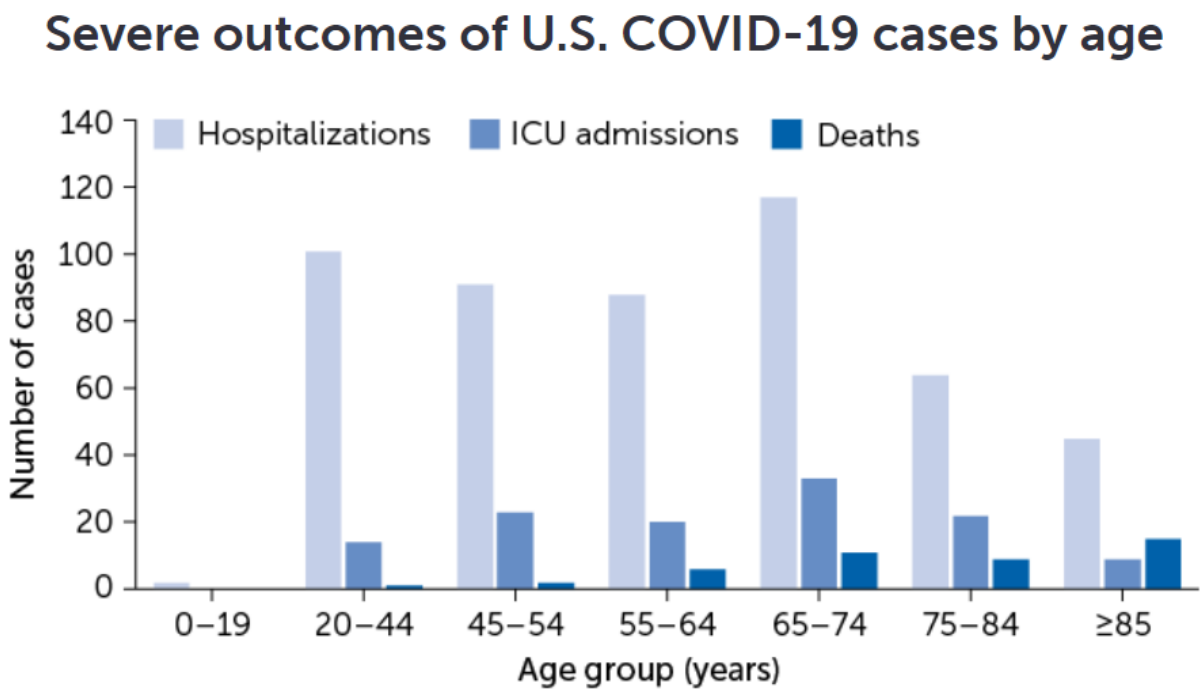
Elderly and Compromised Most at Risk, but Younger People Severely Affected Too

Approximately 1 in 7 patients develop difficulty breathing and other severe complications, requiring hospitalization, while 6 percent become critical. These patients typically suffer failure of the respiratory and other vital systems, and can be susceptible to septic shock, according to a report (pdf) by the World Health Organization. About 10-15 percent of mild-to-moderate patients progress to severe and of those, 15-20 percent progress to critical.

According to the largest study conducted to date in China, COVID-19 patients at the highest risk for poor outcomes include people age 60 and older (especially those over 80, ~15 percent die) and those with underlying conditions, such as hypertension, cancer, diabetes and cardiovascular disease. Those with two chronic conditions were shown to be at a 2.6 greater risk, compared to individuals with none. Nearly 20 percent of COVID-19 patients who had at least one chronic condition had poor outcomes, compared with 4.5 percent of those without any chronic ailments.

An estimated 60 percent of all Americans have at least one chronic health condition, and 40 percent have more than one, putting a large number of people at greater risk of succumbing to COVID-19, particularly if elderly.

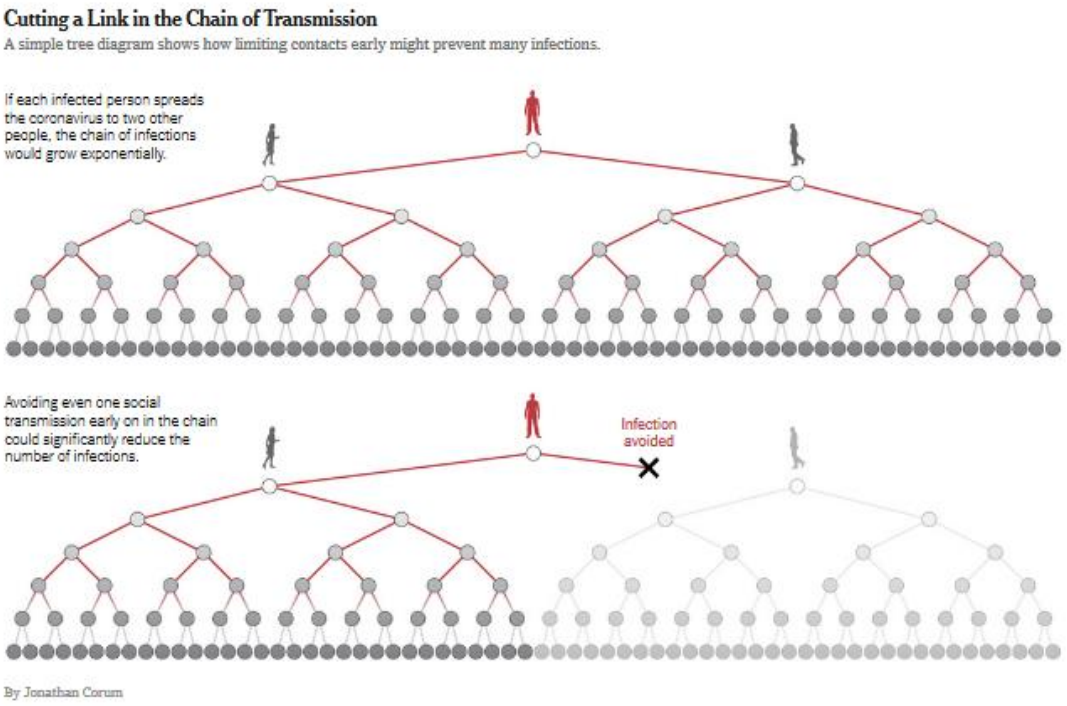
Data compiled by the U.S. Center for Disease Control, however, shows that younger people may also be at risk of severe outcomes. Among hospitalized adults, from February 12 through March 16, 2020, 1 in 5 were 20 to 44 years old, as were 12 percent of those admitted to the ICU. Adults 45 to 64 years old made up 35 percent of hospitalized patients and 36 percent of ICU patients.



Source

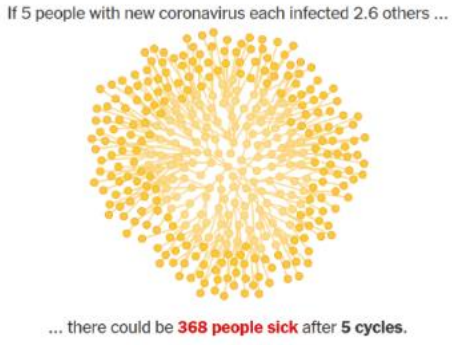
Asymptomatic Transmission

A stealth contagion, the novel coronavirus (SARS-CoV-2) can be shed by people before symptoms arise, lurking up to 14 days in the body before the emergence of any outward signs (e.g., cough, fever, sore throat, shortness of breath) of COVID-19. It is estimated about 85 percent of infected travelers leaving Wuhan, prior to a January 31st lockdown, went undetected while they were still contagious, at which time containing COVID-19 was now near impossible. Outbreaks in other countries invebitably started to appear.



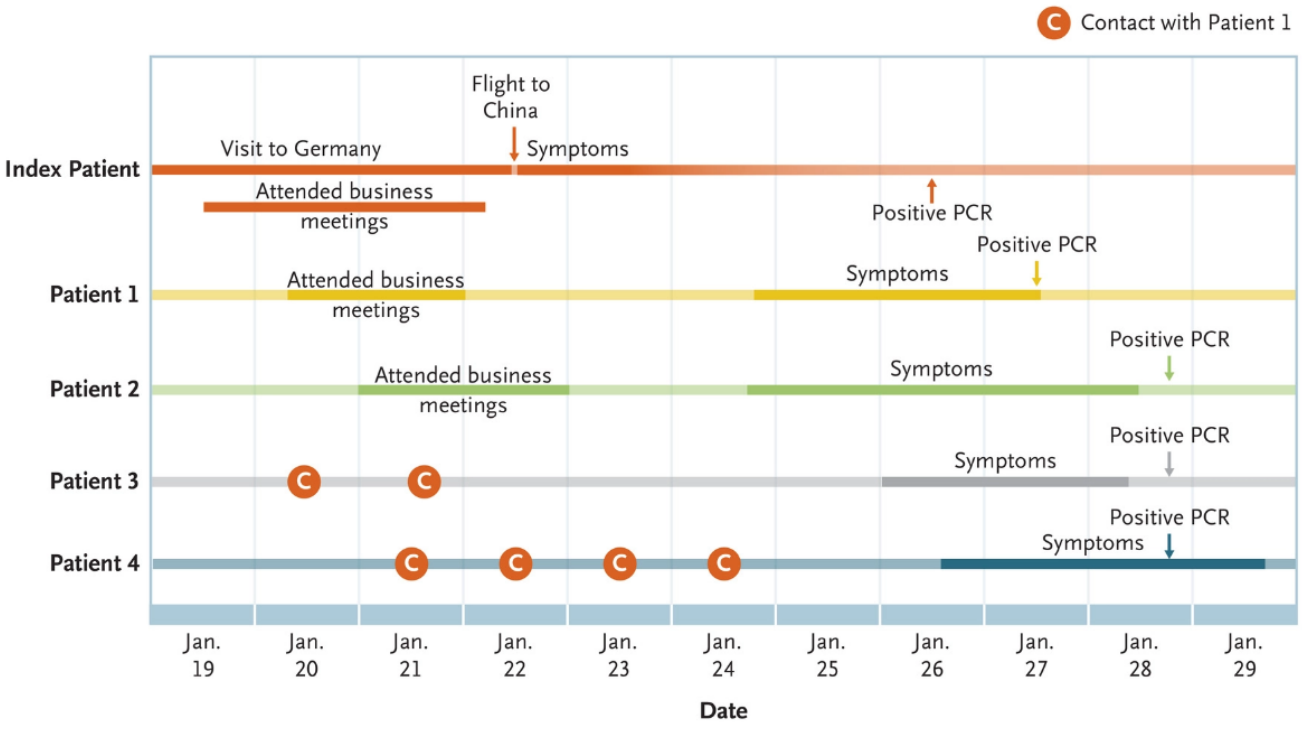
Source

Other transmission dynamics, based on frontline observations by scientists in China:



"[I]n the early stage of the epidemic, the average incubation period was 5.2 days; the doubling time of the epidemic was 7.4 days, i.e., the number of people infected doubled every 7.4 days; the average continuous interval (the average interval time of transmission from one person to another) was 7.5 days; the basic regeneration index (R0) was estimated to be 2.2 to 3.8, meaning that each patient infects 2.2 to 3.8 people on average."

The virus can also remain in the body well-after recovery. According to a study in the *Lancet*, based on research in China, the median length of time the virus stays in the respiratory tract of a patient after symptoms begin is 20 days. Among patients who survived the disease, the virus continued to be shed for between eight and 37 days. SARS-CoV-2 can remain even longer in the stool, for up to 5 weeks after a patient tests negative for the virus based on respiratory samples.



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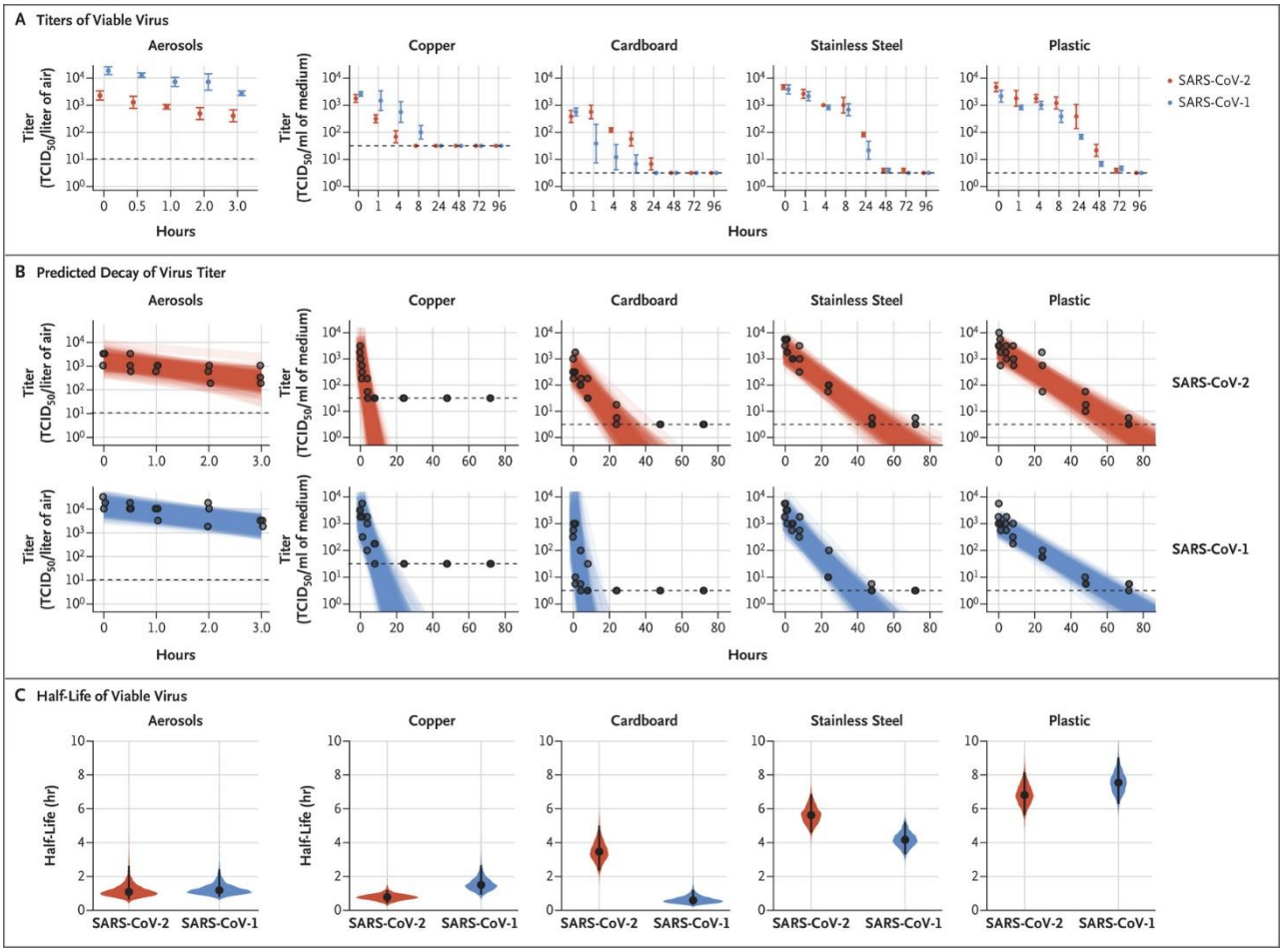
Viability Outside the Body

Experiments (pdf) conducted by Vincent Munster, Chief of the Virus Ecology Section of Rocky Mountain Laboratories, and others in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID), show (see *NEJM* article) the novel coronavirus can stay infectious for days in different environments, depending on the type of surface and the surrounding temperature. Some coronavirus can potentially remain viable—capable of infecting a person—for up to 24 hours on cardboard and up to three days on plastic and stainless steel. When aerosolized into fine, floating particles, the virus remained viable for three hours. On a copper surface, it was four hours. Median length of viability for the virus on stainless steel was 13 hours, and 16 hours on polypropylene, a common type of plastic.

Different environments	Temperature	Survival time
Air	50 ~ 59°F	4 hours
	77°F	2 ~ 3 minutes
Droplets	<77°F	24 hours
Nasal mucus	132.8°F	30 minutes
Liquid	167°F	15 minutes
Hands	68 ~ 86°F	<5 minutes
Non-woven fabric	50 ~ 59°F	<8 hours
Wood	50 ~ 59°F	48 hours
Stainless steel	50 ~ 59°F	24 hours
75% alcohol	Any temperature	<5 minutes
Bleach	Any temperature	<5 minutes

Source



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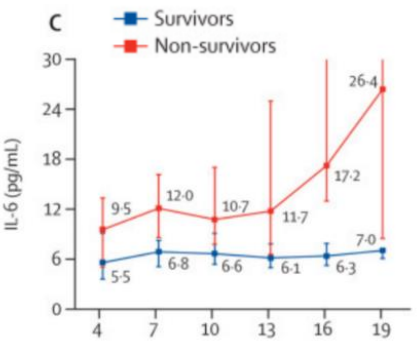
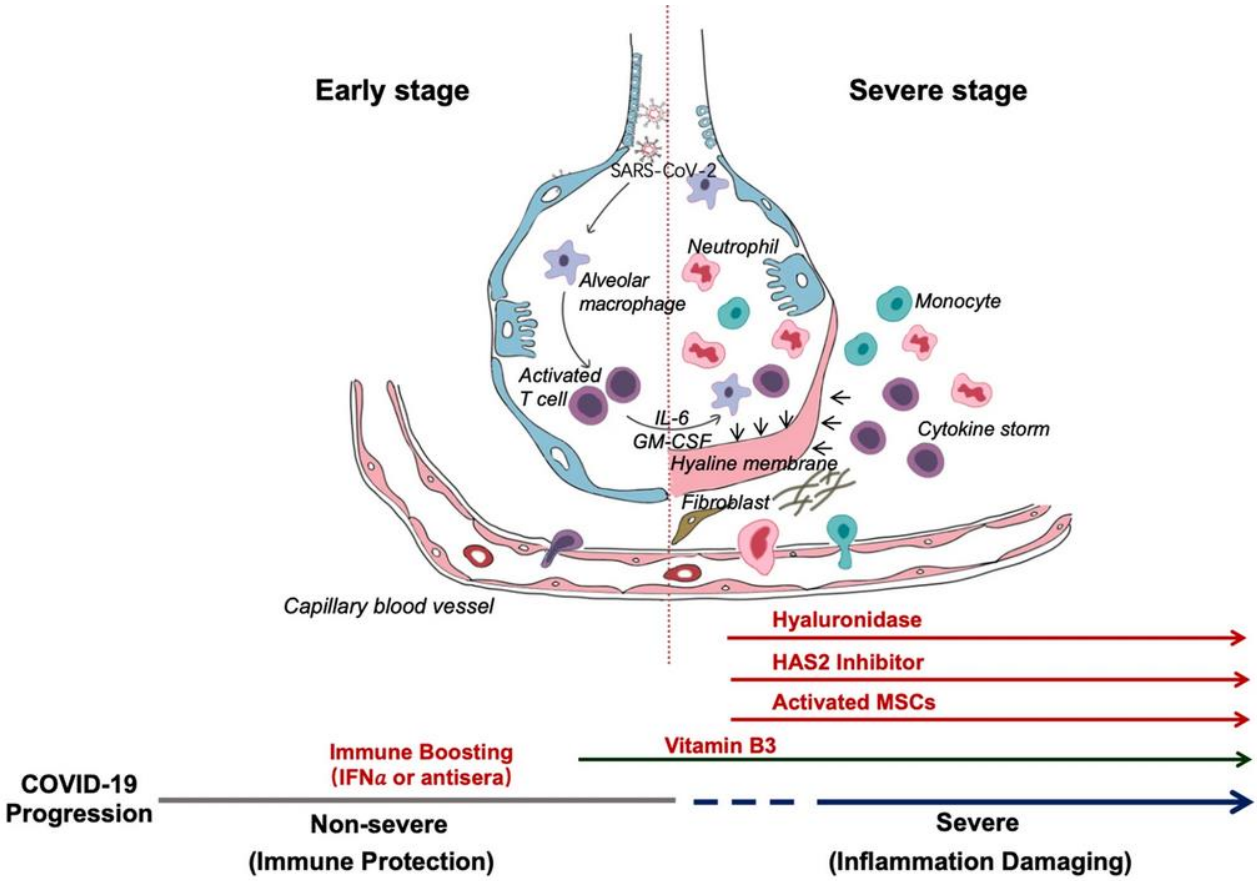
Pathogenesis, Role of IL-6 (Inflammatory Cytokines)

According to recent article in *JAMA*, as well as another article in *Nature*, the pathogenesis of COVID-19 is still not completely understood, though an excessive immune response by the body to fight off the novel coronavirus (triggering a "cytokine storm") and viral evasion of cellular immune responses are thought to play important roles in disease severity. This can lead to Acute Respiratory Distress Syndrome (ARDS)—a leading cause of death among the sickest COVID-19 patients. Scientists at the University of Science and Technology of China (USCT) have identified interleukin 6 (IL-6), a pro-inflammatory cytokine, as the "main culprit" in the body's overreaction when trying to fend off the virus. This has led health authorities in China to recommend Roche's arthritis drug, Actemra (tocilizumab), a monoclonal antibody that inhibits IL-6, to be used in treating patients with COVID-19. A randomized clinical trial evaluating Actemra has commenced, according to Chinese Clinical Trial Registry, with investigators planning to enroll a total of 188 patients—half on Actemra, half on placebo. Regeneron and Sanofi have announced, alongside smaller companies like Tiziana Life Sciences, similar plans to target IL-6 inhibition as a potential novel anti-COVID-19 strategy.



With a 3-in-1 treatment potential—as an antiviral, immuno/anti-inflammatory and antimicrobial drug—Brilacidin might help strengthen the body's innate immune response at early stages of COVID-19, as well as help stem inflammatory reactions and bacterial complications in later stages of the disease.

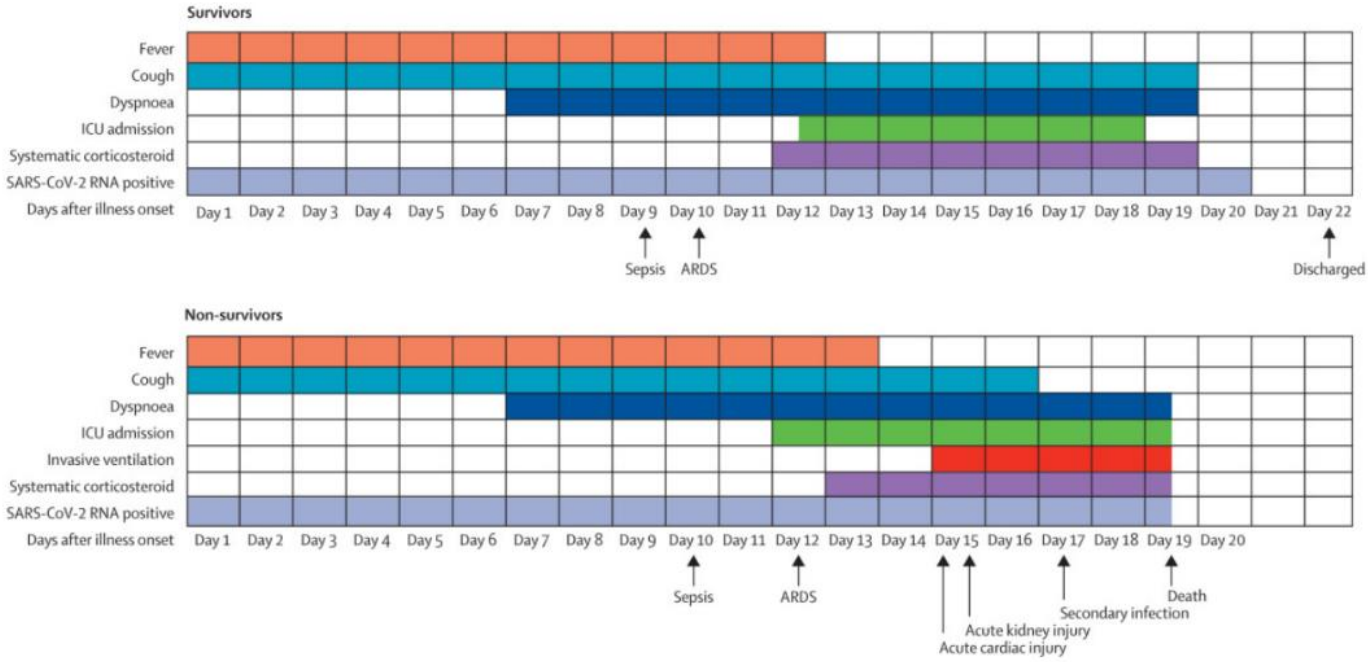


After an incubation period, the invading COVID-19 virus causes non-severe symptoms and elicits protective immune responses. The successful elimination of the infection relies on the health status and the HLA haplotype of the infected individual. In this period, strategies to boost immune response can be applied. If the general health status and the HLA haplotype of the infected individual do not eliminate the virus, the patient then enters the severe stage, when strong damaging inflammatory response occurs, especially in the lungs. At this stage, inhibition of hyaluronan synthase and elimination of hyaluronan can be prescribed. Cytokine activated mesenchymal stem cells can be used to block inflammation and promote tissue reparation. Vitamin B3 can be given to patients starting to have lung CT image abnormalities.

Source

Secondary Infections

Bacterial infections (10-20%) can often co-present in COVID-19 patients. In one retrospective study of 191 patients in China, 95 percent received antibiotics, compared to 21 percent of patients receiving antiviral treatment, 30 percent corticosteroids, and 24 IV immunoglobulin. Half of non-survivors experienced a secondary infection, and ventilator-associated pneumonia occurred in ten (31%) of 32 patients requiring invasive mechanical ventilation. The duration of antibiotic treatment in another study of 99 cases in Wuhan China was between 3 and 17 days, with a median duration of 5 days.



Source

InsideHealthPolicy
An Inside Washington news service

Experts Warn Of Secondary COVID-19 Infections, Antibiotic Resistance

By Kelly Lienhard / March 3, 2020 at 2:59 PM

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Several antimicrobial resistance experts have begun to cite concerns about a possible wave of secondary bacterial and fungal infections in COVID-19 patients that could evolve into antimicrobial-resistant diseases and said the lack of new antibiotic development could exacerbate an already-climbing death rate from the epidemic. The president on Tuesday (March 3) reinstated an advisory council on antibiotic-resistant bacteria coming as stakeholders press Congress to boost incentives for development of new antibiotics. The Antimicrobial Innovation Alliance's top priority on Capitol Hill...

Source

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