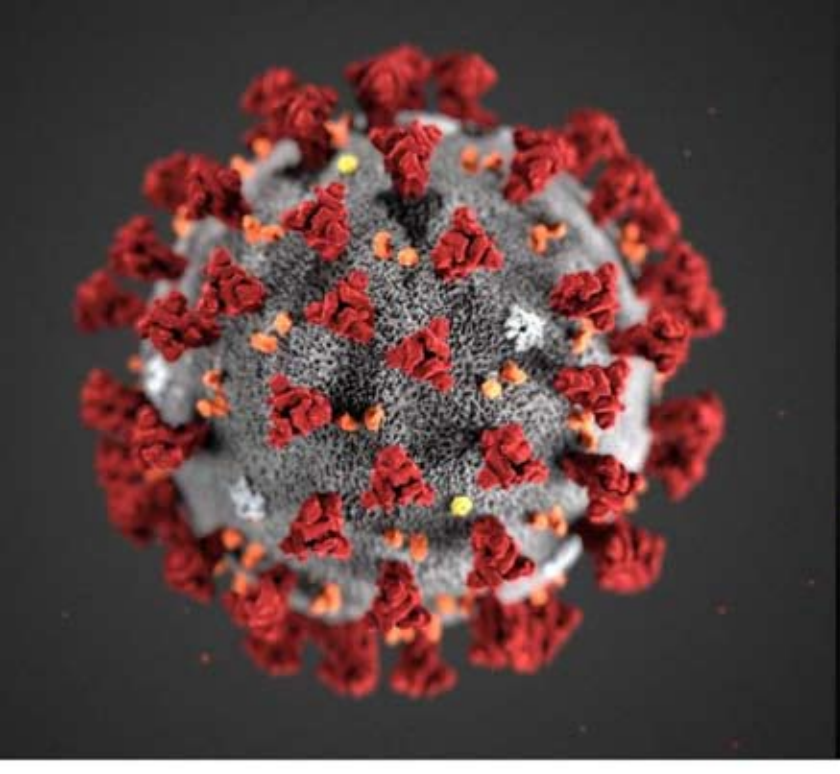


SARS-CoV-2

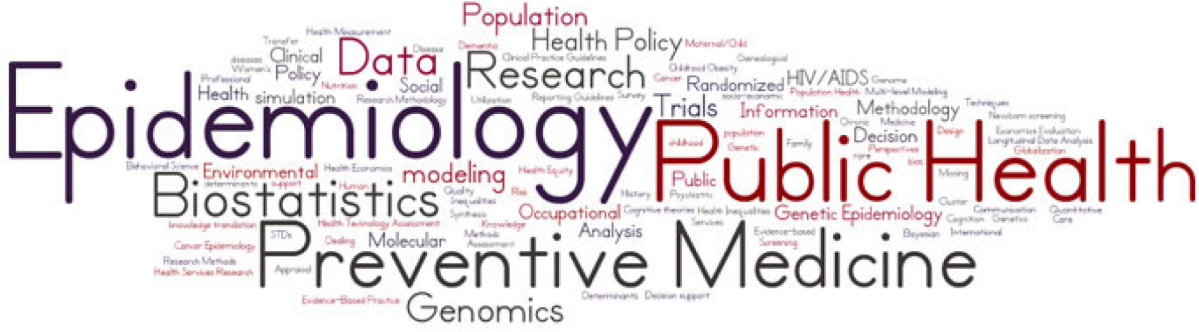
Therapeutic Consideration

CZV2-14+Humic Acid

28 March 2020 w/ Amendments

Presentation Map

- Epidemiology
- Viral Anatomy & Mechanism of Infection
- What is Translational Medicine?
- Humic Acid
- Humic Acid Clinical Studies
- Peptides – Clinical Applications & Product Development
- Real World Data, Toxicity & Cell Proliferation Studies & Future Developments

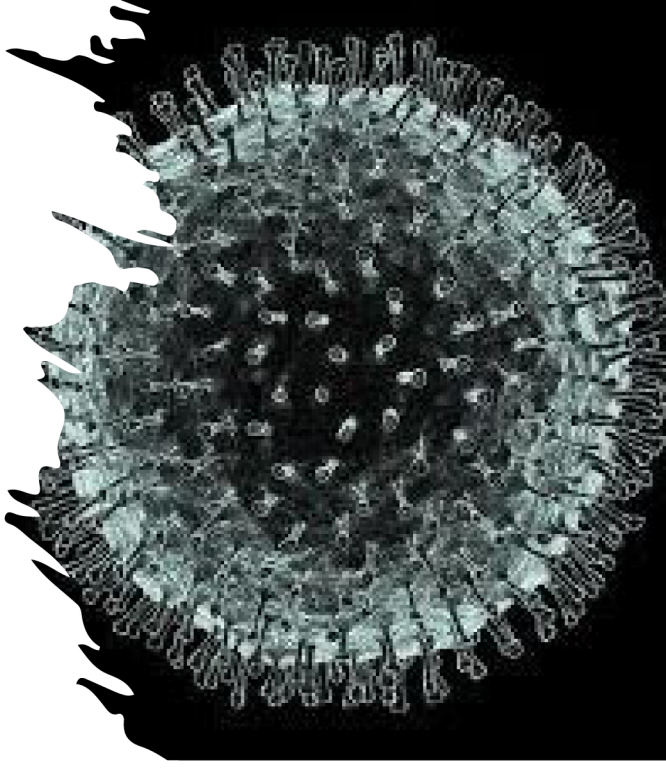

Epidemiology
Public Health
Preventive Medicine
Biostatistics
Data
Research
Population
Health Policy
Clinical
Policy
Health
simulation
Social
Trials
Randomized
HIV/AIDS
Information
Methodology
Decision
Environmental
modeling
Public
Occupational
Genetic Epidemiology
Analysis
Molecular
Genomics
Health Measurement
Transfer
diseases
Women's
Professional
Nutrition
Research Methodology
Disease
Dementia
Clinical Practice Guidelines
Maternal/Child
Genealogical
Childhood Obesity
Cancer
Population Health
Multi-level Modeling
Genome
Techniques
Newborn screening
Economics Evaluation
Longitudinal Data Analysis
Globalization
Design
Medicine
Chronic
Perspectives
bias
Missing
Cluster
Communication
Cognition
Genetics
Quantitative
Care
International
Bayesian
Evidence-based
Screening
Utilization
Reporting Guidelines
Survey
Quality
Inequalities
Synthesis
Risk
Health Equity
Mastery
Psychiatric
Cognitive theories
Health Inequalities
Services
Methods
Assessment
Knowledge
Health Economics
Human
Behavioral Science
determinants
support
knowledge translation
Health Technology Assessment
STDs
Dealing
Cancer Epidemiology
Research Methods
Health Services Research
Appraisal
Evidence-Based Practice
Determinants
Decision support
childhood
population
Genetic
Family
more
Life course epidemiology

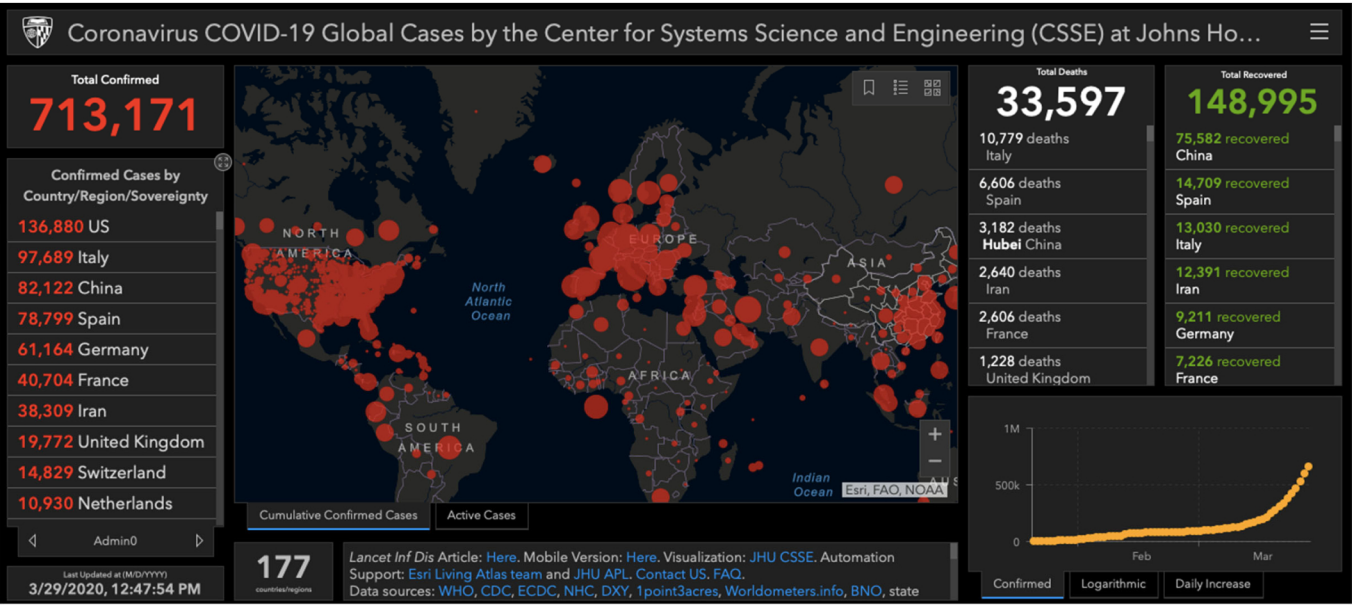
Johns Hopkins University's Center for Systems Science and Engineering

COVID-19 Prevalence Map

Boston Children's Hospital

Health Map




Coronavirus COVID-19 Global Cases by the Center for Systems Science and Engineering (CSSE) at Johns Ho…
Total Confirmed
713,171
Confirmed Cases by Country/Region/Sovereignty
136,880 US
97,689 Italy
82,122 China
78,799 Spain
61,164 Germany
40,704 France
38,309 Iran
19,772 United Kingdom
14,829 Switzerland
10,930 Netherlands
Admin0
Last Updated at (M/D/YYYY)
3/29/2020, 12:47:54 PM
NORTH AMERICA
EUROPE
ASIA
AFRICA
SOUTH AMERICA
North Atlantic Ocean
Indian Ocean
Esri, FAO, NOAA
Cumulative Confirmed Cases
Active Cases
177
countries/regions
Lancet Inf Dis Article: Here. Mobile Version: Here. Visualization: JHU CSSE. Automation Support: Esri Living Atlas team and JHU APL. Contact US. FAQ.
Data sources: WHO, CDC, ECDC, NHC, DXY, 1point3acres, Worldometers.info, BNO, state
Total Deaths
33,597
10,779 deaths Italy
6,606 deaths Spain
3,182 deaths Hubei China
2,640 deaths Iran
2,606 deaths France
1,228 deaths United Kingdom
Total Recovered
148,995
75,582 recovered China
14,709 recovered Spain
13,030 recovered Italy
12,391 recovered Iran
9,211 recovered Germany
7,226 recovered France
1M
500k
0
Feb
Mar
Confirmed
Logarithmic
Daily Increase

WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020

Good afternoon.

In the past two weeks, the number of cases of COVID-19 outside China has increased 13-fold, and the number of affected countries has tripled.

There are now more than 118,000 cases in 114 countries, and 4,291 people have lost their lives.

Thousands more are fighting for their lives in hospitals.

In the days and weeks ahead, we expect to see the number of cases, the number of deaths, and the number of affected countries climb even higher.

WHO has been assessing this outbreak around the clock and we are deeply concerned both by the alarming levels of spread and severity, and by the alarming levels of inaction.

We have therefore made the assessment that COVID-19 can be characterized as a pandemic.

Pandemic is not a word to use lightly or carelessly. It is a word that, if misused, can cause unreasonable fear, or unjustified acceptance that the fight is over, leading to unnecessary suffering and death.

Describing the situation as a pandemic does not change WHO's assessment of the threat posed by this virus. It doesn't change what WHO is doing, and it doesn't change what countries should do.

We have never before seen a pandemic sparked by a coronavirus. ***This is the first pandemic caused by a coronavirus****.*

And ***we have never before seen a pandemic that can be controlled****, at the same time.*

WHO has been in full response mode since we were notified of the first cases.

And we have called every day for countries to take urgent and aggressive action.

We have rung the alarm bell loud and clear.

Asymptomatic Transmission?

- "There is also strong evidence that it can be transmitted by people who are just mildly ill or even presymptomatic. That means COVID-19 will be much harder to contain than the Middle East respiratory syndrome or severe acute respiratory syndrome (SARS), which were spread much less efficiently and only by symptomatic people,"
- Bill Gates, NEJM, 2.28.2020

- "Asymptomatic and mildly symptomatic transmission are a major factor in transmission for Covid-19," said Dr. William Schaffner, a professor at Vanderbilt University School of Medicine and longtime adviser to the CDC. "They're going to be the drivers of spread in the community."
- CNN, 3.14.2020

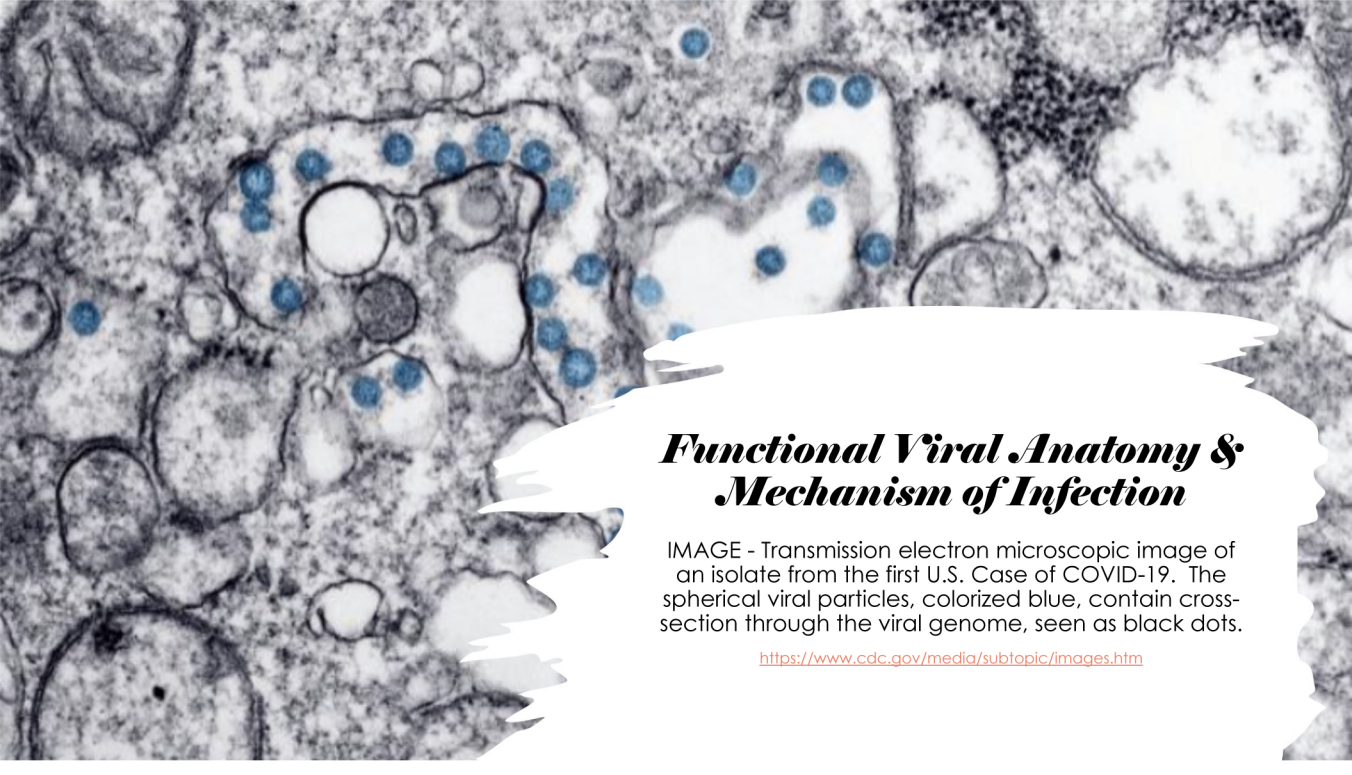
Former CDC director: There's a long war ahead and our Covid-19 response must adapt

Dr. Tom Frieden

Updated 9:33 AM ET, Sat March 21, 2020

- The virus is much more infectious than influenza or the SARS virus, which it closely resembles. This week, new data showed that SARS-CoV-2, the virus that causes Covid-19, can live on contaminated surfaces as the SARS virus can, so it may spread, sometimes explosively, from doorknobs, elevator buttons and contaminated surfaces in hospitals and elsewhere. But we also learned that, unlike SARS, patients become highly infectious before they become seriously ill, explaining at least in part why Covid-19 acts like a super-SARS, far more infectious than its vanquished cousin.

- It's not just older people with underlying conditions who become very ill and can die. Younger adults, previously healthy people and some children develop viral pneumonia. Although prior reports suggested that 80% of people got only mild disease, it now appears that about half of these people, despite not needing hospital admission, have moderately severe pneumonia, which can take weeks or longer to recover from.



Functional Viral Anatomy & Mechanism of Infection

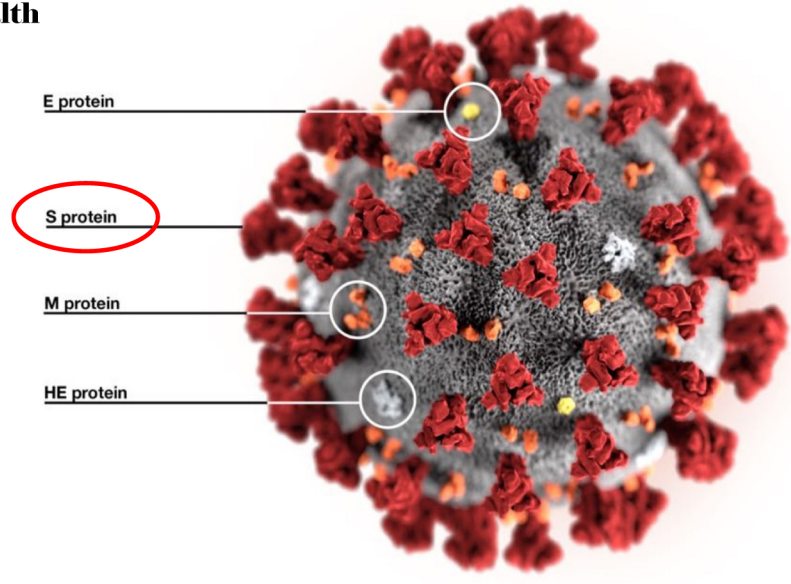
IMAGE - Transmission electron microscopic image of an isolate from the first U.S. Case of COVID-19. The spherical viral particles, colorized blue, contain cross-section through the viral genome, seen as black dots.

https://www.cdc.gov/media/subtopic/images.htm

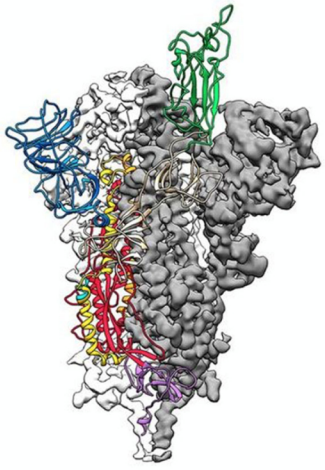
Anatomy of SARS-Cov-2, “COVID-19”

National Institutes of Health

Spike = surface glycoprotein



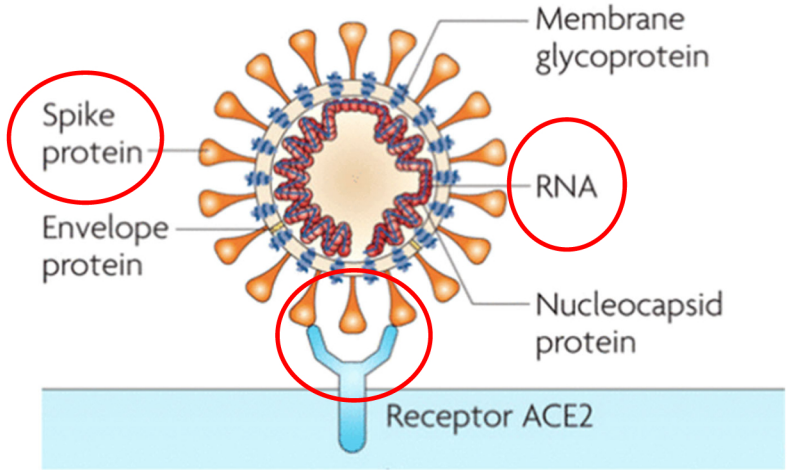
Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh L, Abiona O, Graham BS, McLellan JS, Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2000 Feb 19.
doi: 10.1126/science.abb2507



- Spike protein
  - Green = receptor binding domain

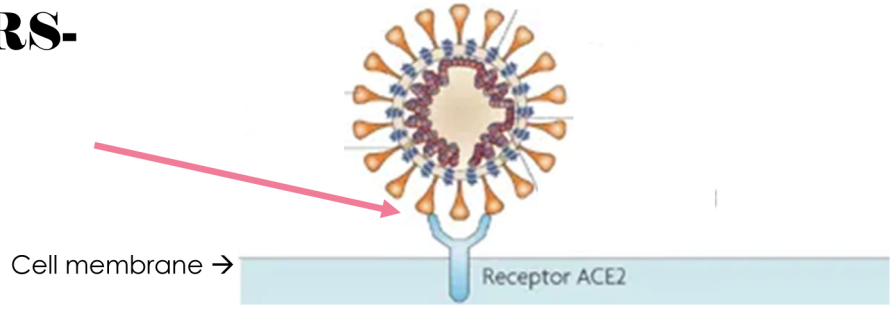
- Supplementary Materials; https://science.sciencemag.org/cgi/content/full/science.abb2507/DC1

Anatomy of SARS-Cov-2, "COVID-19"



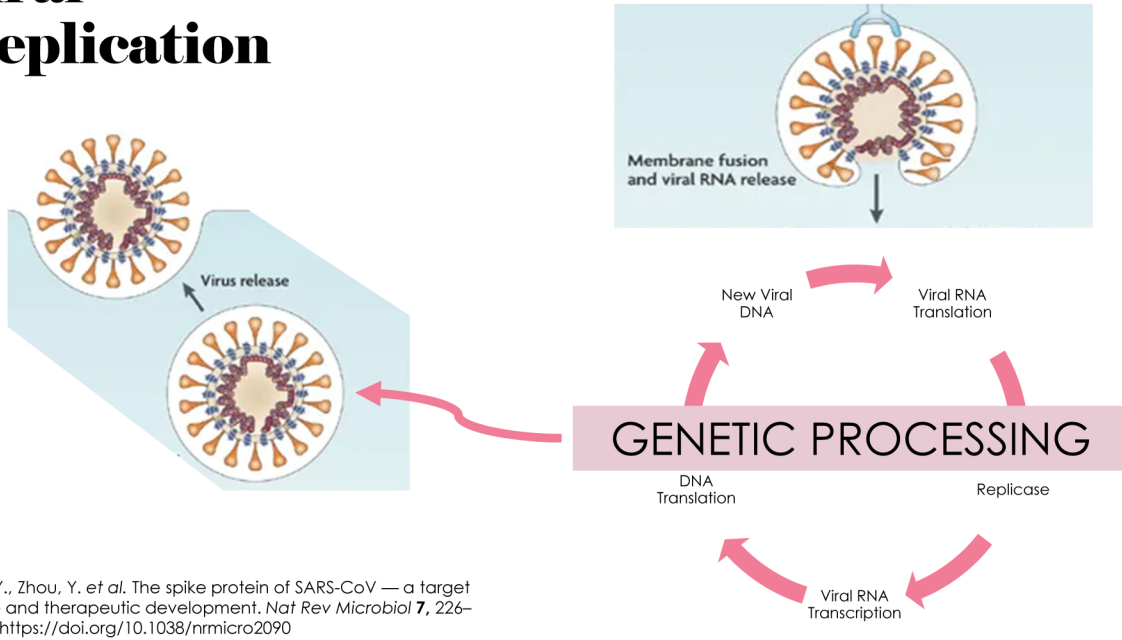
Research and Development on Therapeutic Agents and Vaccines for COVID-19 and Related Human Coronavirus Diseases; Cynthia Liu, Qiongqiong Zhou, Yingzhu Li, Linda V. Garner, Steve P. Watkins, Linda J. Carter, Jeffrey Smoot, Anne C. Gregg, Angela D. Daniels, Susan Jervey, and Dana Albaiu. *ACS Central Science* **Article ASAP.** DOI: 10.1021/acscentsci.0c00272

Mechanism of Infection – SARS-CoV



Du, L., He, Y., Zhou, Y. *et al.* The spike protein of SARS-CoV — a target for vaccine and therapeutic development. *Nat Rev Microbiol* **7,** 226–236 (2009). https://doi.org/10.1038/nrmicro2090

Viral Replication

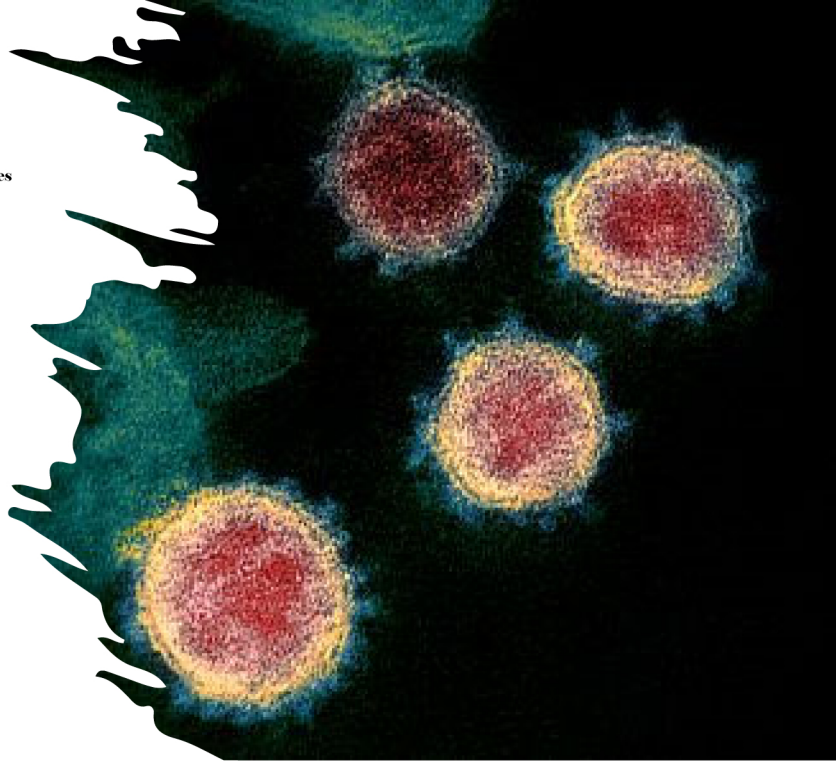


Du, L., He, Y., Zhou, Y. *et al.* The spike protein of SARS-CoV — a target for vaccine and therapeutic development. *Nat Rev Microbiol* **7,** 226–236 (2009). https://doi.org/10.1038/nrmicro2090

Mechanism of Infection

Novel coronavirus structure reveals targets for vaccines and treatments
National Institutes of Health
March 3, 2020

- Surface proteins (spikes or glycoproteins) protrude from surface
- Spikes latch onto human cells and then undergo a structural change that allows the viral membrane to fuse with the cell membrane
- Spikes bind to receptors on human cell surface called angiotensin-converting enzyme 2.



Novel coronavirus structure reveals targets for vaccines and treatments

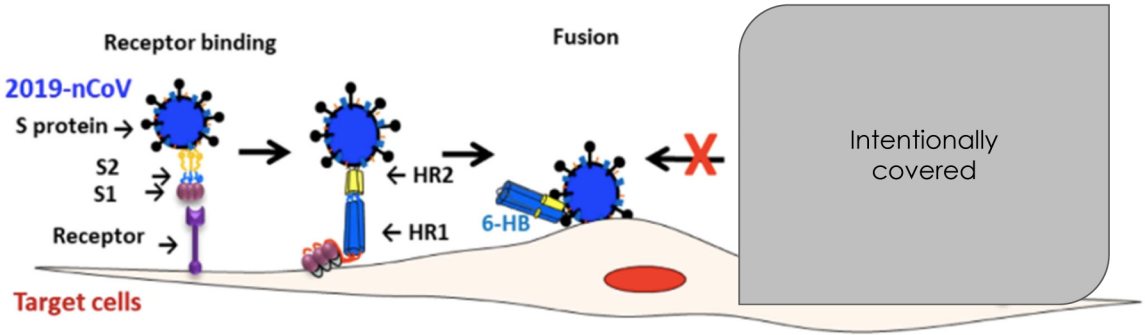
National Institutes of Health, March 3, 2020

- SARS-CoV-2 spike (S-glycoprotein) is 10-20x more likely to bind ACE2 on human cells than the spike from the 2002 SARS virus.

This may explain one reason why Covid-19 spreads more easily than SARS in 2002.

- Reference:
  - **2002 – 2004: Severe Acute Respiratory Syndrome (SARS)**
  - 2012: Middle East Respiratory Syndrome (MERS)

Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein



The putative antiviral mechanism of 2019-nCoV-HR2P and EK1. After binding of RBD in S1 subunit of 2019-nCoV S protein to the potential receptor ACE2 on the host cell, S2 subunit changes conformation by inserting **Fusion Peptide** into the cell membranes and triggering the association between the HR1 and HR2 domains to form 6-HB, which brings the viral and cellular membranes in close proximity for fusion

Xia, S., Zhu, Y., Liu, M. *et al.* Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. *Cell Mol Immunol (2020 Feb 11)*. https://doi.org/10.1038/s41423-020-0374-2

An introduction to Translational Medicine

Volume 1, Issue 1, March 2016

BIOENGINEERING & TRANSLATIONAL MEDICINE

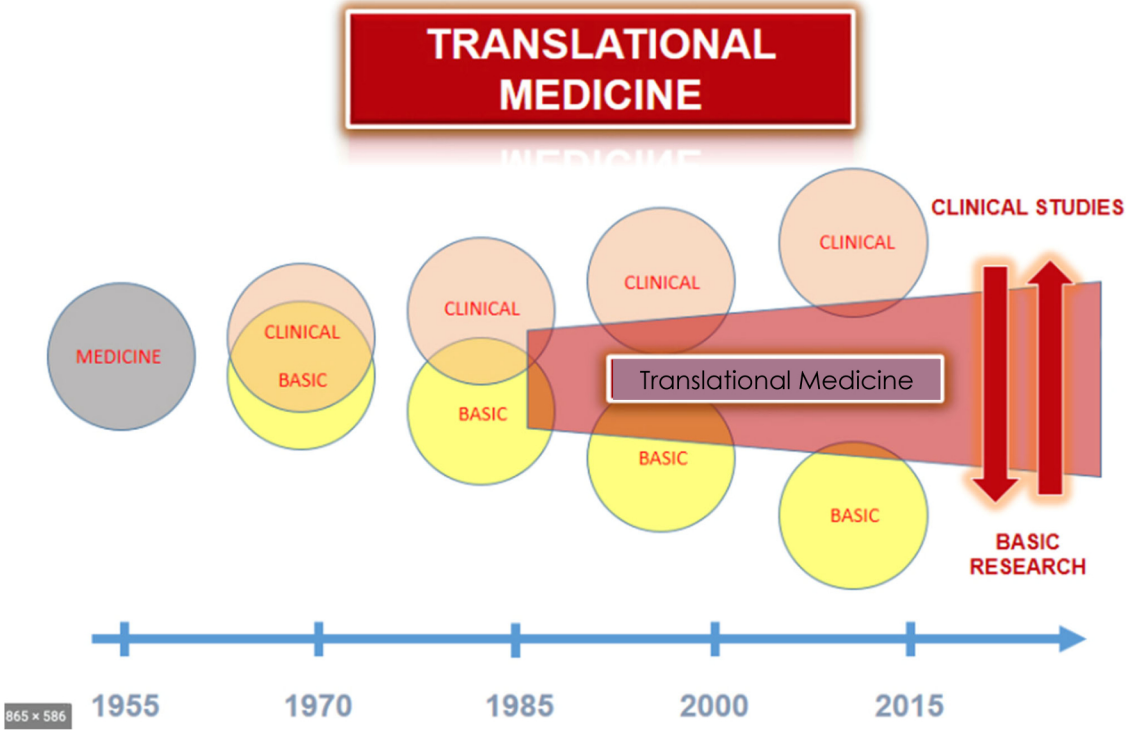
Open Access

An official publication of the American Institute of Chemical Engineers

Society for Biological Engineering

AIChE The Global Home of Chemical Engineers

WILEY


TRANSLATIONAL MEDICINE
CLINICAL STUDIES
MEDICINE
CLINICAL
BASIC
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BASIC
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BASIC
Translational Medicine
BASIC RESEARCH
1955
1970
1985
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2015
865 × 586

Translational Medicine

Translational medicine is collaborative bench-to-bedside-to-bench research that aims to further understand disease biology and identify the patient populations most likely to derive benefit from therapy.

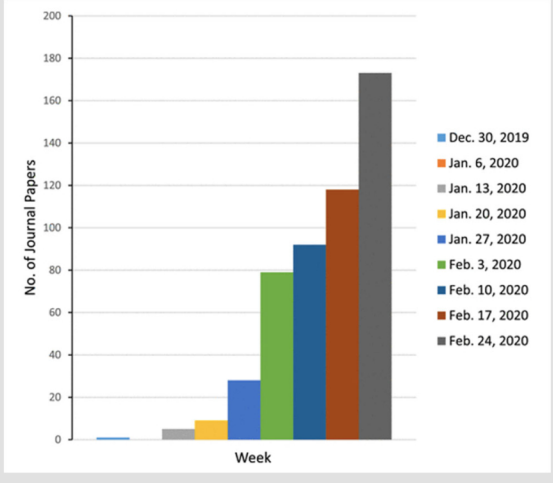
Successful translational medicine requires nimble collaboration and a fluid exchange of information across discovery, clinical development and commercialization.

In translational medicine, experimentation and exploratory research is fundamental to form hypotheses that can be tested in the clinic. This research aims to dissect the human pathophysiology of disease to generate insights that inform our clinical approach.

https://www.bms.com/assets/bms/us/en-us/pdf/translational-medicine-infographic.pdf

Number of journal articles related to COVID-19 published each week.

- **Research and Development on Therapeutic Agents and Vaccines for COVID-19 and Related Human Coronavirus Diseases;** Cynthia Liu, Qiongqiong Zhou, Yingzhu Li, Linda V. Garner, Steve P. Watkins, Linda J. Carter, Jeffrey Smoot, Anne C. Gregg, Angela D. Daniels, Susan Jervey, and Dana Albaiu. *ACS Central Science* **Article ASAP.** DOI: 10.1021/acscentsci.0c00272



Translational Medicine

Real World Data: Promise & Pitfalls

- Real-world data (RWD) are health-related information reported and collected in real-world medical settings.
- RWD analyses generate insights about a medicine's effectiveness, safety and associated costs
- Robust RWD analyses typically use advanced statistical methods designed to address confounding factors. **Failure to use appropriate methods can lead to RWD studies that generate incorrect or unreliable conclusions.**

https://www.bms.com/life-and-science/science/5-facts-real-world-data.html

HUMIC ACID

HISTORY & MEDICAL APPLICATIONS

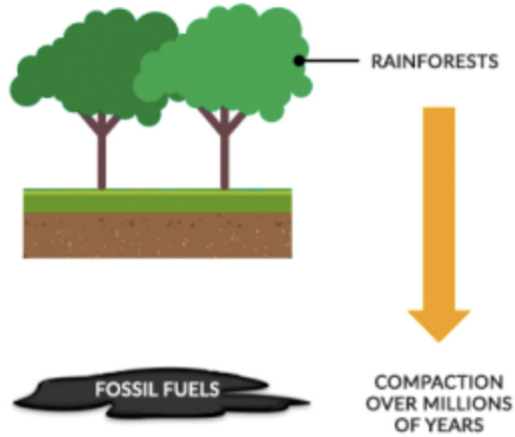
Humic Acids

- 1761: Walerius named soil deposits of composed organic matter - **humin.**
- 1786: First study published on humic acid
- Applications
  - Industrial – oil & gas industry
  - Agriculture - fertilizer
- Robust history in Ayurvedic Medicine

Origin of humic acid

- Deposits of decomposed plant materials that originated 280 million years ago, in the **Paleozoic coal age**
- Water, micro-organisms (e.g. bacteria) and time fuel changes in soil chemistry that drive the formation of deposits

Carboniferous Rainforest Collapse



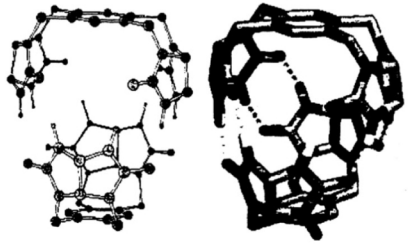
When sourced and processed, humic acid inhibits viral fusion

Sourcing is very important

Humic Acid – a flexible polyfunctional molecular system

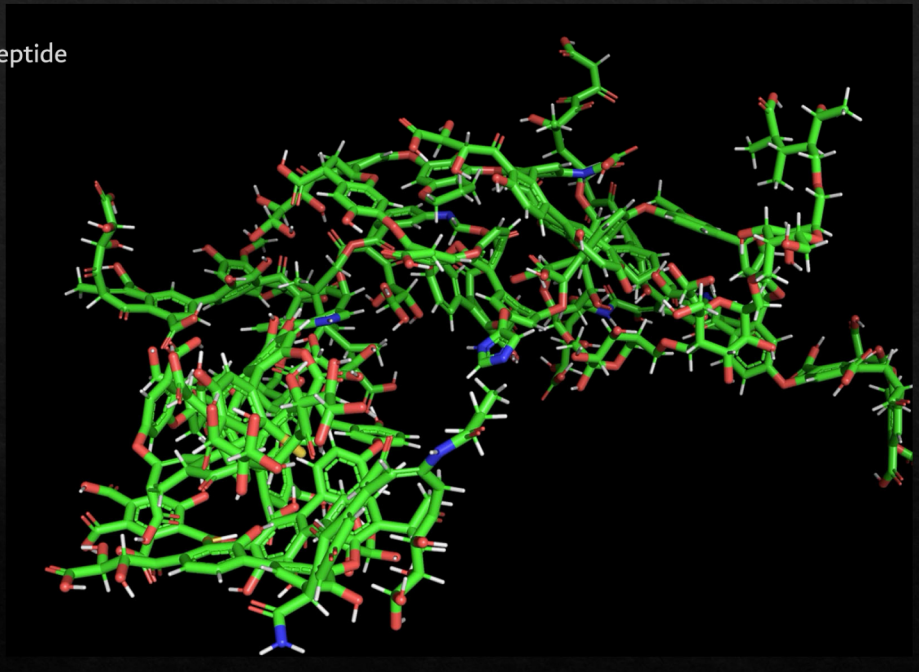
Bruccoleri AG, Sorenson BT, Langford CH. **Molecular Modeling of Humic Structures**. Department of Chemistry, University of Calgary

Orsi, M. **Molecular dynamics simulation of humic substances**. *Chem. Biol. Technol. Agric.* **1**, 10 (2014). https://doi.org/10.1186/s40538-014-0010-4



- Existing models
  - fail to account for the different energy states of a molecule. This promotes a false impression of reality.
  - Fail to consider molecular interaction with water

Humic Acid – 3D model, no peptide



Product Efficacy Overview - Humic Acid, *in vitro* study

Anti-Viral Activity

HIV-1, HIV-2, HSV-1, HSV-2, CMV, EBV, Influenza, Hemorrhagic Fevers

- University of Southern California
- Southern Research Institute
- Specialty Laboratory
- National Institute of Health

Product Efficacy Overview - Humic Acid, *in vitro* study

Anti-Viral Activity – HIV-1

- University of Southern California
  - Complete inhibition of viral replication
    - Dose: 25 mcg/mL
    - Comparable AZT dose: 50 mcg/mL, with cytostasis

- Southern Research Institute
  - Prophylactic activity
    - Prevents HIV-1 infection of multiple cell lines
    - Therapeutic in chronically infected cells
    - Inhibits HIV-1 viral attachment
      - IC50 = 0.01 – 0.88 mcg/mL in HeLa CD4 LTR B-gal cells

Product Efficacy Overview - Humic Acid, *in vitro* study

Anti-Viral Activity – HSV 1 & 2

- Specialty Laboratory
  - (+) Prophylactic against infection
    - Prior to cell contact with virus
    - When applied immediately at time of contact
  - (+) Therapeutic after viral infection

Product Efficacy Overview - Humic Acid, *in vitro* study

Anti-Viral Activity

Herpes, CMV; VZV; EBV; Influenza, Hemorrhagic Fevers

- National Institute of Health, Bethesda, Maryland
  - Non-toxic
  - Anti-viral activity greatest against influenza & hemorrhagic fever strains
  - Mechanism of action – **inhibition of viral fusion**

Broad Spectrum Antiviral Effectiveness of Natural and Synthetic Humates
Virology Branch, Antiviral Research & Antimicrobial Chemistry Program, Division of Microbiology & Infectious Diseases
Screening & Testing Program for Antiviral, Immunomodulatory, Anti-tumor and/or Drug Delivery Activities, National Institutes of Allergy & Infectious Diseases, National Institute of Health (NIH), August 9, 2002

- Endpoints:
  - **TC50** – toxic concentration of drug that results in 50% cell toxicity
  - **CP50** – concentration of drug that results in 50% decrease in cell proliferation
  - **IC50** – inhibitory concentration of drug that prevents infection in 50% of cells treated
  - **IC90** – inhibitory concentration of drug that prevents infection in 90% of cells treated

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Table III. Effective Inhibitory Concentrations at 50% (IC_{50}) and 90% (IC_{90}) of Humate Materials and Acyclovir (ACV) Reference Compound with Herpes Simplex Virus Type 1 (HSV-1) (HFF Cells)

Humate	IC_{50}, µg/mL	IC_{90}, µg/mL
CA	6	17.3
CGA	15.1	–
HA	4.7	13.1
HGA	16.9	51.6
Acyclovir	1.2–1.6	7.9

Table IV. Effective Inhibitory Concentrations at 50% (IC_{50}) and 90% (IC_{90}) of Humate Materials and Acyclovir (ACV) Reference Compound with Herpes Simplex Virus Type 2 (HSV-2) (HFF Cells)

Humate	IC_{50}, µg/mL	IC_{90}, µg/mL
CA	6.2	–
CGA	4.4	–
HA	2.5	6.7
HGA	2.1	19.7
Acyclovir	1.1–1.3	9.5

Broad Spectrum Antiviral Effectiveness of Natural and Synthetic Humates
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Table VI. Effective Inhibitory Concentrations at 50% (IC_{50}) and 90% (IC_{90}) of Humate Materials and Acyclovir (ACV) Reference Compound with Varicella Zoster Virus (VZV) (HFF Cells)

Humate	IC_{50}, µg/mL	IC_{90}, µg/mL
CA	>100	>100
CGA	>100	>100
HA	53.5	85.8
HGA	24	47.2
Acyclovir	0.23–0.38	16.3

Table VII. Effective Inhibitory Concentrations at 50% (IC_{50}) and 90% (IC_{90}) of Humate Materials and Acyclovir (ACV) Reference Compound with Epstein-Barr Virus (EBV) (Daudi Cells)

Humate	IC_{50}, µg/mL	IC_{90}, µg/mL
CA	>0.4	>0.4
CGA	21.1	33
HA	>50	>50
HGA	16.8	49
Acyclovir	1.8–2.4	16.3

Broad Spectrum Antiviral Effectiveness of Natural and Synthetic Humates

Virology Branch, Antiviral Research & Antimicrobial Chemistry Program, Division of Microbiology & Infectious Diseases

Screening & Testing Program for Antiviral, Immunomodulatory, Anti-tumor and/or Drug Delivery Activities, National Institutes of Allergy & Infectious Diseases, National Institute of Health (NIH), August 9, 2002

Table VIII. Effective Inhibitory Concentrations at 50% (IC_{50}) and 90% (IC_{90}) of Humate Materials and Ribavirin Reference Compound with Influenza Virus Type A (New Caledonia/20/99) (H1N1) (MDCK Cells)

Humate	IC_{50}, µg/mL CPE Method	IC_{50}, µg/mL NR Method	IC_{50}, µg/mL VY Method	IC_{90}, µg/mL
CA	1	0.6	3.2	4
CGA	45	40	–	–
HA	2.5	2.5	3.2	5
HGA	3.7	3.2	–	–
Ribavirin	0.55	0.38	0.32	1.4

Table IX. Effective Inhibitory Concentrations at 50% (IC_{50}) and 90% (IC_{90}) of Humate Materials and Ribavirin Reference Compound with Influenza Virus Type A (Panama/2007/99) (H3N2) (MDCK Cells)

Humate	IC_{50}, µg/mL CPE Method	IC_{50}, µg/mL NR Method	IC_{50}, µg/mL VY Method	IC_{90}, µg/mL
CA	<1	<1	0.4	0.5
CGA	6	6.5	–	–
HA	<1	<1	0.22	0.4
HGA	4.5	3.2	–	–
Ribavirin	1.3	1.8	1.9	1.4

Table X. Effective Inhibitory Concentrations at 50% (IC_{50}) of Humate Materials and Ribavirin Reference Compound with Influenza Virus Type A (NWS/33) (H1N1) (MDCK Cells)

Humate	IC_{50}, µg/mL CPE Method	IC_{50}, µg/mL NR Method	IC_{50}, µg/mL VY Method
CA	0.65-1	0.55-0.85	–
CGA	–	–	–
HA	1.3	1.3	–
HGA	18	17	–
Ribavirin	5-6.0	4.6-6.5	–

Table XI. Effective Inhibitory Concentrations at 50% (IC_{50}) of Humate Materials and Ribavirin Reference Compound with Influenza Virus Type A (PR/8/34) (H1N1) (MDCK Cells)

Humate	IC_{50}, µg/mL CPE Method	IC_{50}, µg/mL NR Method	IC_{50}, µg/mL VY Method
CA	8.5	10	–
CGA	–	–	–
HA	14	18	–
HGA	18	18	–
Ribavirin	9	12	–

Broad Spectrum Antiviral Effectiveness of Natural and Synthetic Humates
Virology Branch, Antiviral Research & Antimicrobial Chemistry Program, Division of Microbiology & Infectious Diseases
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Table XIX. Effect of Intraperitoneal Treatment of Synthetic Humate CA[a] on Influenza Virus Type A (Shangdong/09/93) (H3N2) Infection in Mice[b]

		Toxicity Controls		Infected Treated Mice		
Treatment[c]	Dosage, mg/kg/day	Survivors/ Total	Mean Host Weight Change[d], g	Survivors/ Total	Mean Day to Death[e] ± SD	Mean Day 11 SaO_2, % ± SD
Humate CA	50	3/3	-0.8	0/10	6.5 ± 4.2	76.4 ± 4.6
	25	3/3	-0.7	3/10[f]	10.3 ± 4.8	79.8 ± 4.6
	12.5	3/3	0.2	4/10[g]	10.2 ± 2.8	81.7 ± 4.9[f]
Ribavirin	75	3/3	0.1	10/10[h]	>21.0 ± 0.0[h]	88.2 ± 1.6[h]
Saline	–	–	–	0/20	9.2 ± 3.5	76.9 ± 3.8
Normal Controls	–	3/3	0.6	5/5	>21.0 ± 0.0	89.4 ± 2.2

[a] Diluent: sterile saline. [b] Female BALB/c mice, 18-21 g. [c] Treatment schedule: bid x 5 beg; 4-h pre-virus exposure. Experiment duration: 21 days. [d] Difference between initial weight and weight 18 hours after final treatment. [e] Mean day to death of mice dying prior to day 21. [f] $P<0.05$; [g] $P<0.01$; [h] $P<0.001$, compared to saline-treated controls.

Broad Spectrum Antiviral Effectiveness of Natural and Synthetic Humates

Virology Branch, Antiviral Research & Antimicrobial Chemistry Program, Division of Microbiology & Infectious Diseases

Screening & Testing Program for Antiviral, Immunomodulatory, Anti-tumor and/or Drug Delivery Activities, National Institutes of Allergy & Infectious Diseases, National Institute of Health (NIH), August 9, 2002

Results

All humates evaluated were not cytotoxic at levels at least as high as 100 µg/mL, as shown below in **Table I**. Visual observation of synthetic humate CA and natural-product humate HA with uninfected MDCK cells in toxicity control wells appeared initially to indicate drug toxicity. However, the drugs were not in fact toxic as revealed by Neutral Red assays. Rather, the humate materials were found to bind to cell surfaces, thereby changing their color and giving them an exanimate appearance. This discoloration was observed in a concentration-dependent manner at levels where antiviral activity was present.

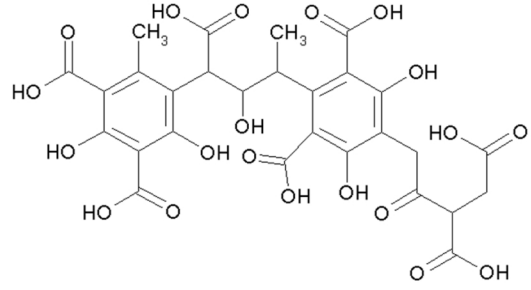
Table I. Toxic Concentrations at 50% (TC_{50}) of Humate Materials with Indicated Cell Lines

	TC_{50}, µg/mL					
				LLC-MK₂[d]		
Humate	BSC-1[a]	HFF[b]	MDCK[c]	Trial 1	Trial 2a[f]	Trial 2b[g]
CA	>100	>100	>100	>100	>1000	>1000
CGA	>100	>100	–[e]	–[e]	>1000	>1000
HA	>100	>100	>100	>100	>1000	>1000
HGA	>100	>100	–[e]	–[e]	700	>1000

[a] African green monkey kidney cells. [b] Human foreskin fibroblast cells. [c] Madin Darby canine kidney cells. [d] Adult rhesus monkey kidney cells. [e] Not evaluated. [f] Neutral Red assay. [g] Visual assay.


clinical
trial
medical
research
test
science
health
vaccine
laboratory
plastic
experiment
genome
modern
medicine
technician
glasses
protection
professional
scientist
work
microbiology
outbreak
pharmaceutical
recording
female
control
biochemistry
sample
disease
treatment
statistics
virus
liquid
biology
discovery
cancer
medication
pharmacist
applied
viral
oncology
tools
improvement
knowledge
chemistry
tech
bio
preclinical
disposable
blood
man
physiology
ebola
illness
tube
results
woman
corporate
care
lab
human
job
biologist
company
clinic
safety
analysis
business
medium
trials
chemist

Amar S, Escovar Y., A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Pilot Study to Investigate the Effects of Humic Acid on Symptoms of Influenza, 2 Sept 2018



Humic Acid
Characteristics

Anti-inflammatory

Anti-viral

- Rhinovirus (Sydow *et al.*, 1986)
- HIV (Laub, 1995 & 2000)
- HSV Types 1 & 2 (Helbig, *et al.*, 1987, Kloeckring, 1991)
- Influenza Types A & B (Laub, 2000, Sydow *et al*, 1986)

Anti-bacterial

Safe

- Up to 50mg/kg (Schiller *et al.*, 1979)

Humic Acid
Mechanism of Action

Primary

- **Inhibits viral fusion**
  - Prevents the attachment of viral particles to host cells (Laub, 1999)
  - De facto inhibition of viral replication

Secondary

- **Inhibits viral RNA synthesis**
  - Inhibits endonuclease activity of viral RNA polymerase (2)

2) Lu FJ, Tseng SN, et al. In Vitro Anti-Influenza Virus Activity of Synthetic Humate Analogues Derived from Protocatechuic Acid. Arch. Virol. 2002, 147(2), 273-284

Pharmacodynamics

- Humic acid's affinity for viral antigens is greater than viral affinity for cell surface receptors.
- Humic acid can reverse viral binding.

Humic Acid & Influenza

Study Design

Participants

- Goal: 40
- Randomized: 37
- Completed: 33

Dose

- 500 mg or placebo
- 3x day
- 14 days

Touchpoints

- Baseline
- Day 7
- Day 14

Assessments

- Subjective
- Performance Metric - VAS
- Vitals
- Biomarkers

Amar S, Escovar Y., **A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Pilot Study to Investigate the Effects of Humic Acid on Symptoms of Influenza**, 2 Sept 2018

Humic Acid & Influenza

Symptom Endpoints

Fever & chills

Myalgias & arthralgias

Headache

Fatigue

Nasal congestion & rhinorrhea

Earache

Cough

Amar S, Escovar Y., **A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Pilot Study to Investigate the Effects of Humic Acid on Symptoms of Influenza**, 2 Sept 2018

Humic Acid & Influenza

Biomarkers of Interest

- Complete Blood Count
  WBC, differential
- T-lymphocytes
  CD4+, CD8+
- Inflammatory Markers
  TNF-a & IL-8
- Serum chemistry and liver enzymes

Amar S, Escovar Y., **A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Pilot Study to Investigate the Effects of Humic Acid on Symptoms of Influenza**, 2 Sept 2018

Humic Acid & Influenza

Subject Parameters

Permissions

Anti-pyretics

Expectorants

Throat losenges

Exclusions

No acetaminophen, aspirin, ilbuprofen and other NSAIDS **4 hours before temperature reading**

Amar S, Escovar Y., **A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Pilot Study to Investigate the Effects of Humic Acid on Symptoms of Influenza**, 2 Sept 2018

Humic Acid & Influenza

Results – Symptom Scores

Symptom Scores

- Greater reduction in HA group than control
- Not statistically significant
- HA cohort - *greater symptom severity at baseline, (+) smokers*

Percent Improvement

Symptom	HA Group	Placebo
Cough	61.9	36.8
Fever	91.7	81.8
Myalgia/arthralgia	86.4	62.5
Chills	91.7	66.7
Fatigue	80.0	54.5
Rhinorrhea	66.7	62.5

Amar S, Escovar Y., **A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Pilot Study to Investigate the Effects of Humic Acid on Symptoms of Influenza**, 2 Sept 2018

Humic Acid & Influenza

Results - Visual Analog Scale (VAS)

- **Statistically significant** increase in VAS scores in both test groups, with *greater increases found from baseline to Week 2 in HA Group compared to Placebo*
- HA Group - greater severity of symptoms at baseline, with lower VAS scores

VAS Improvement

p < 0.001 – all subgroups

	HA Group *n = 19*	Placebo *n = 18*
Week 1	64%	54%
Week 2	107%	76%
Progress Week 1 to 2	+43%	+22%
40-60 y/o	164%	70.3%

Amar S, Escovar Y., **A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Pilot Study to Investigate the Effects of Humic Acid on Symptoms of Influenza**, 2 Sept 2018

Humic Acid & Influenza

Results: TNF-α and IL-8

TNF-α

$p = 0.027$

	HA Group	Placebo
Week 2	-26.7%	-7.1%

- Observation: TNF levels were lower across the study in the HA Group

IL-8

	HA Group	Placebo
Week 2	-3.2%	-10.2%

- Recall: IL-8 stimulates activation of T-cells & monocytes.

Amar S, Escovar Y., **A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Pilot Study to Investigate the Effects of Humic Acid on Symptoms of Influenza**, 2 Sept 2018

Humic Acid & Influenza

Results: CD4+ and CD8+

CD4+

	HA Group	Placebo
Week 2	+2.8%	-3.1%

CD8+

	HA Group	Placebo
Week 2	+1.4%	-1.1%

- Observation: Net change between HA Group and placebo of ~6% suggests that HA may have some role in modulating the immune response.

Amar S, Escovar Y., **A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Pilot Study to Investigate the Effects of Humic Acid on Symptoms of Influenza**, 2 Sept 2018

Humic Acid & Influenza

Results – Adverse Events

- Adverse events
  - Check numbers
- Elevated AST/ALT
  - 2 HA Group
  - 1 Placebo
  - Causation - Unknown

	HA Group	Placebo
Diarrhea	3	2
Dizziness	1	0
Nausea	1	0
Vomiting	1	0
Stomach ache / discomfort	2	0

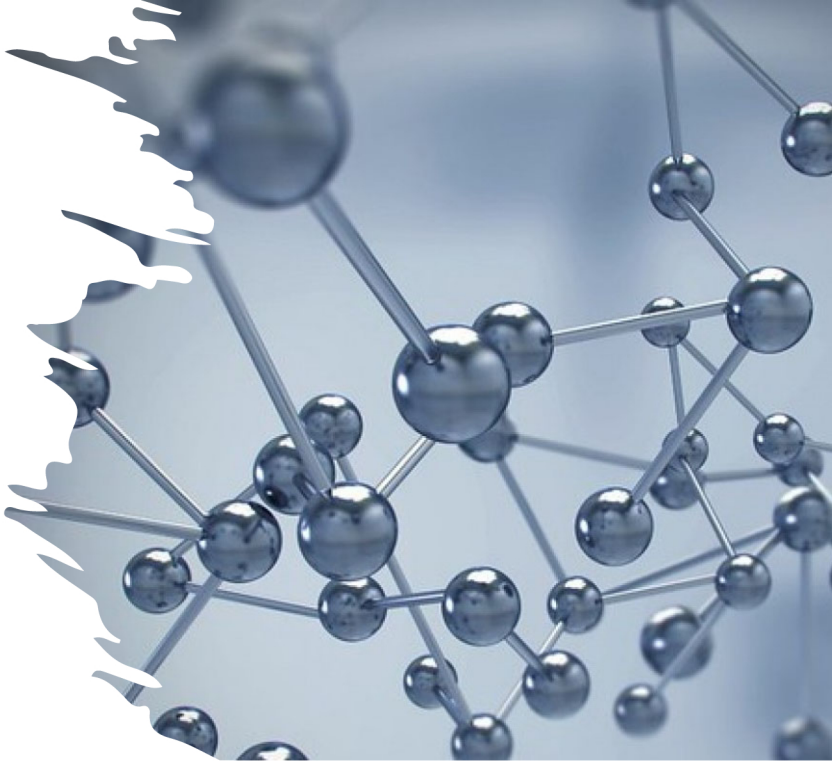
Amar S, Escovar Y., **A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Pilot Study to Investigate the Effects of Humic Acid on Symptoms of Influenza**, 2 Sept 2018

PEPTIDES

Clinical applications

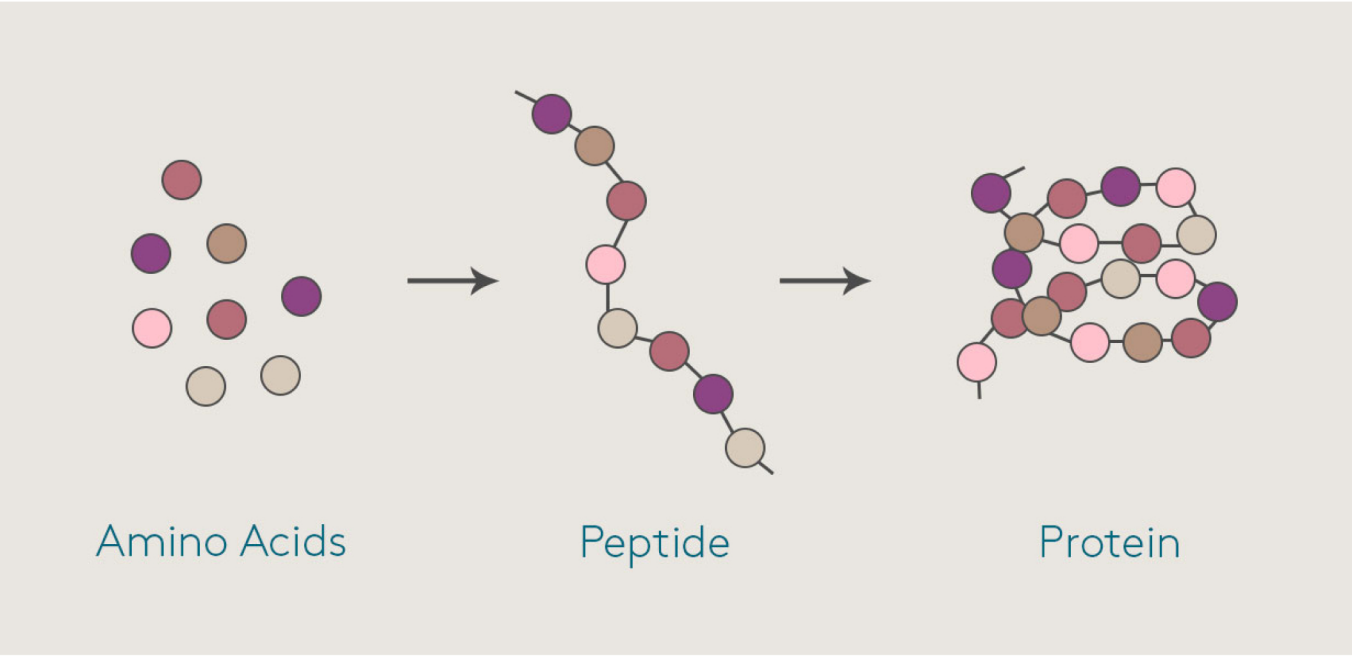
&

Product development



What is a peptide?

- Two or more amino acids linked together
- Typically between 2 and 50 amino acids
- Protein > 50 amino acids

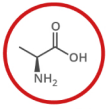
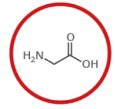
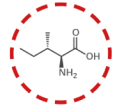
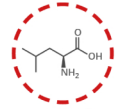
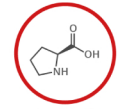
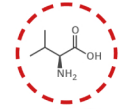
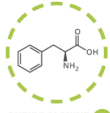
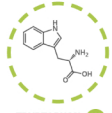
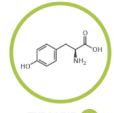
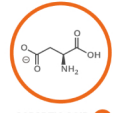
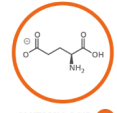
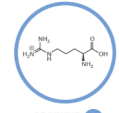
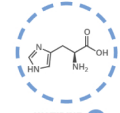
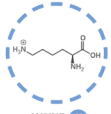
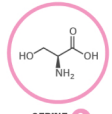
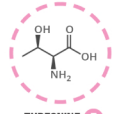
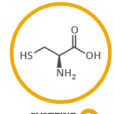
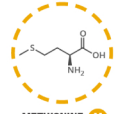
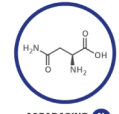
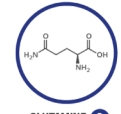

Amino Acids
Peptide
Protein

A GUIDE TO THE TWENTY COMMON AMINO ACIDS

AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.

Chart Key: ALIPHATIC · AROMATIC · ACIDIC · BASIC · HYDROXYLIC · SULFUR-CONTAINING · AMIDIC · NON-ESSENTIAL · ESSENTIAL

Chemical Structure — single letter code → NAME (A) — three letter code

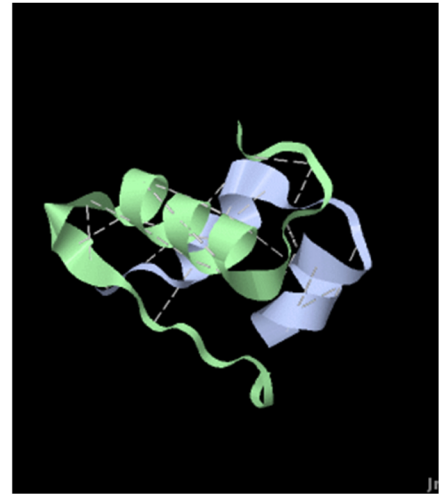
 ALANINE (A) *ala*	 GLYCINE (G) *gly*	 ISOLEUCINE (I) *ile*	 LEUCINE (L) *leu*	 PROLINE (P) *pro*	 VALINE (V) *val*	
 PHENYLALANINE (F) *phe*	 TRYPTOPHAN (W) *trp*	 TYROSINE (Y) *tyr*	 ASPARTIC ACID (D) *asp*	 GLUTAMIC ACID (E) *glu*	 ARGININE (R) *arg*	 HISTIDINE (H) *his*
 LYSINE (K) *lys*	 SERINE (S) *ser*	 THREONINE (T) *thr*	 CYSTEINE (C) *cys*	 METHIONINE (M) *met*	 ASPARAGINE (N) *asn*	 GLUTAMINE (Q) *gln*

Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.

Peptides move biology

INSULIN

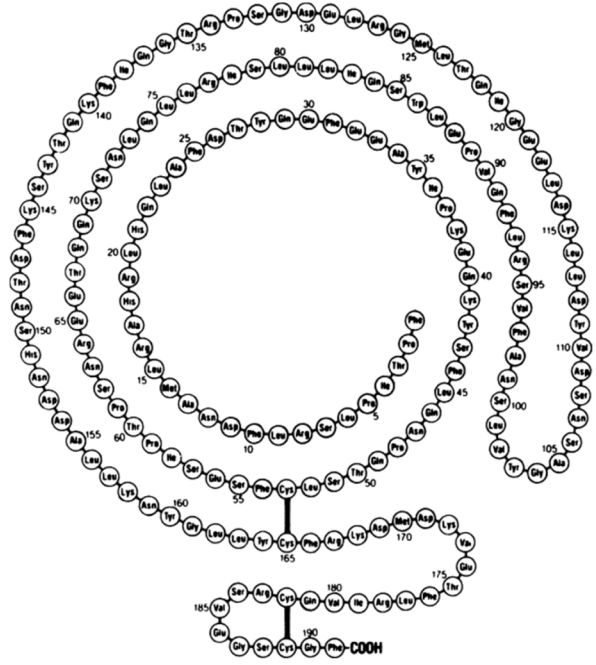
- Insulin is a small protein consisting of **two peptide chains**. The A chain consists of 21 amino acid residues, and the B chain — of 30 amino acids.
- The chains are connected by two disulfide bonds.



Growth Hormone

191 amino acid sequence

http://www.rcsb.org/3d-view/1HGU



Peptides – Other Examples

Neurotrophs – Cerebrolysin & Dihexa

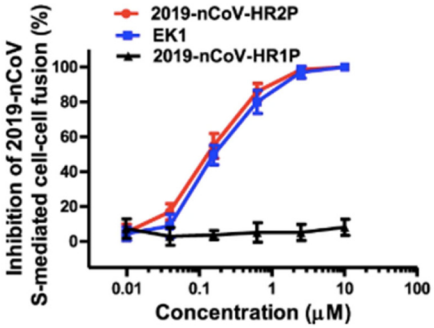
Immune Support – Thymosins

Skin Rejuvenation – cosmeceutical products

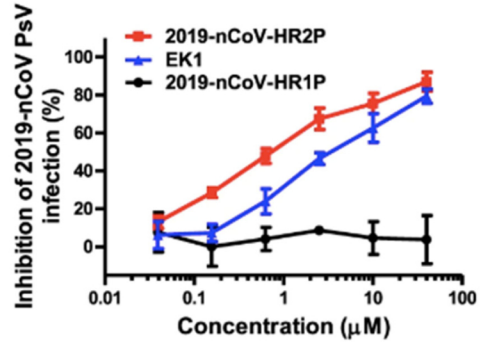
Bioregulators – Epitalon

Proof of Concept - Xia, S., Zhu, Y., Liu, M. et al. Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. Cell Mol Immunol (2020 Feb 11). https://doi.org/10.1038/s41423-020-0374-2

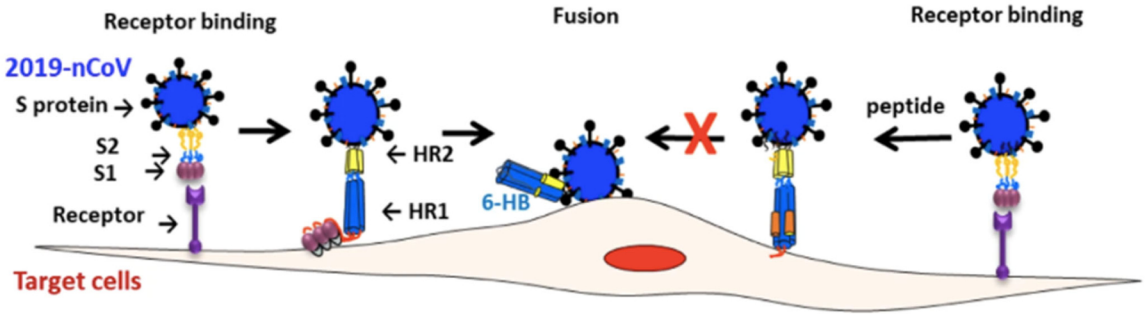
Inhibitory activity of peptides on 2019-nCoV S-mediated cell–cell fusion.



Inhibition of peptides on pseudotyped 2019-nCoV infection.



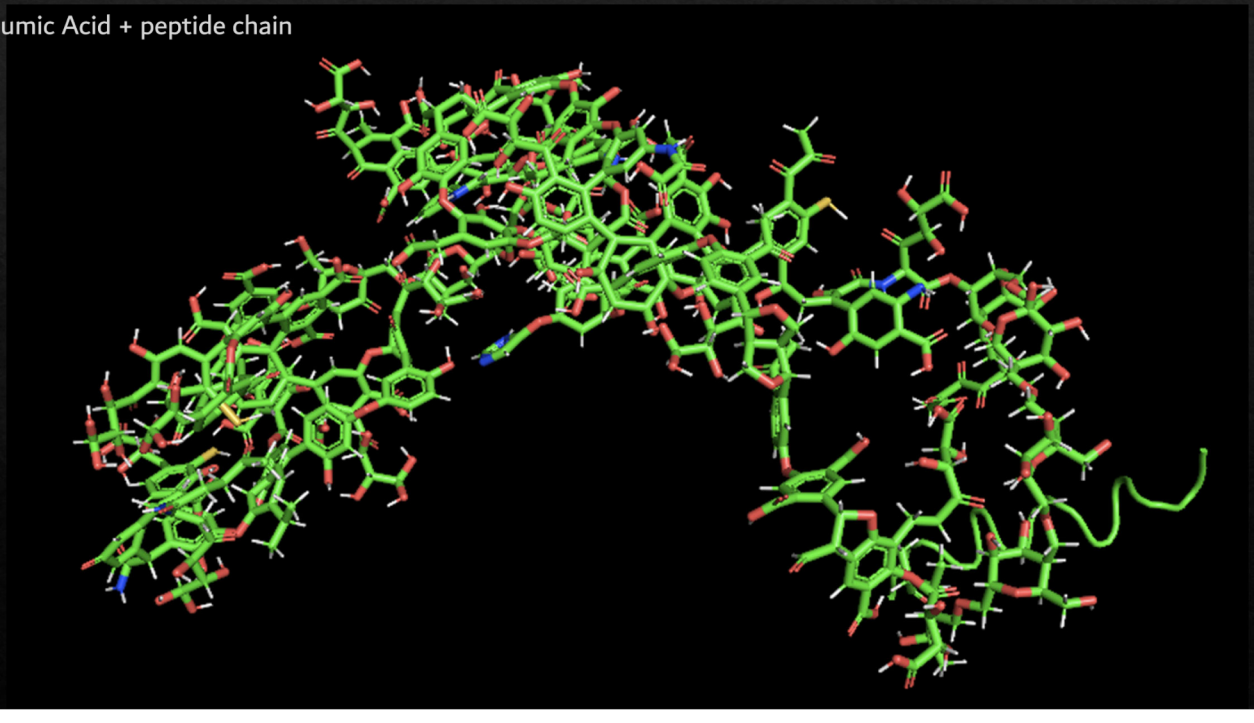
REVIEW: Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein



The putative antiviral mechanism of 2019-nCoV-HR2P and EK1. After binding of RBD in S1 subunit of 2019-nCoV S protein to the potential receptor ACE2 on the host cell, S2 subunit changes conformation by inserting **Fusion Peptide** into the cell membranes and triggering the association between the HR1 and HR2 domains to form 6-HB, which brings the viral and cellular membranes in close proximity for fusion (left part of **k**). *In the presence of 2019-nCoV-HR2P or EK1 peptide, three copies of the peptide bind to the 2019-nCoV S-HR1-trimer to form heterologous 6-HB, thus blocking the formation of viral homologous 6-HB and thus* ***inhibiting viral and cell membrane fusion*** *(right part of image)*

Xia, S., Zhu, Y., Liu, M. *et al.* Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. *Cell Mol Immunol (2020 Feb 11*). https://doi.org/10.1038/s41423-020-0374-2

Humic Acid + peptide chain



Fusion

Humic acid with peptide

- 14 amino acid sequence
- Binds to spike protein (S-glycoprotein)
- Anchors humic acid to virus
- Clinical trial, 10 patients
- **Patient outcome data better than humic acid alone**

In vitro evaluation of the anti-viral properties of humic acid and polypeptide and its investigation of mechanism of action. Internal Study

- Results
  - **Viricidal**
    - HSV-1 – 100% by day 3
    - HSV-2 – 60% by day 3
  - **Viral Attachment Inhibition**
    - CMV – 100% by day 3
    - RSV-2 -100% by day 3

- Method
  - Antiviral activity – plaque assay & virus yield assay

A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Pilot Study to Investigate the Effects of Humic Acid + Peptide on Symptoms of Influenza, Internal document

Protocol mirrors from HA control study, 2018

Participants: 20

Treatment group: 10

Placebo group: 10

Drop-outs: 0

Humic Acid + Peptide - Influenza Results

Results - Symptom Scores

PERCENT IMPROVEMENT

Symptom	HA Group *n = 19*	Placebo *n = 18*	HA + Peptide *n = 10*	Placebo n = 10
Cough	61.9	36.8	79.2	32.8
Fever	91.7	81.8	93.4	79.2
Myalgia/arthralgia	86.4	62.5	90.5	60.7
Chills	91.7	66.7	92.6	59.5
Fatigue	80.0	54.5	83	51
Rhinorrhea	66.7	62.5	68.9	59.5

Humic Acid + Peptide & Influenza

Results: % change, TNF-α and IL-8

TNF-α

	HA + Peptide *n = 10*	Placebo *n = 10*
Week 2	-47.1%	-1.6%

	HA Group *n = 19*	Placebo *n = 18*
Week 2	-26.7%	-7.1%

IL-8

	HA + Peptide *n = 10*	Placebo *n = 10*
Week 2	-35%	8.3%

	HA Group *n = 19*	Placebo *n = 18*
Week 2	-3.2%	-10.2%

Humic Acid + Peptide & Influenza

Results - Visual Analog Scale

- **Augmented improvement** in VAS scores noted in HA+Peptide group vs. HA alone.
- Recall: HA Group ONLY - greater severity of symptoms at baseline, with lower VAS scores

	HA + Peptide *n = 10*	Placebo *n = 10*
Week 1	82.7%	35.9%
Week 2	120.8%	65.5%
Progress Week 1 to 2	+38.1%	+29.6%
40-60 y/o	--- %	--- %

VAS Improvement; p < 0.001 – all subgroups

	HA Group *n = 19*	Placebo *n = 18*
Week 1	64%	54%
Week 2	107%	76%
Progress Week 1 to 2	+43%	+22%
40-60 y/o	164%	70.3%

Humic Acid + Peptide & Influenza

Results: % change, CD4+ and CD8+

CD4+

	HA + Peptide *n = 10*	Placebo *n = 10*
Week 2	73.9%	-3.1%

	HA Group *n = 19*	Placebo *n = 18*
Week 2	+2.8%	-3.1%

CD8+

	HA + Peptide *n = 10*	Placebo *n = 10*
Week 2	-5.1%	-0.6%

	HA Group *n = 19*	Placebo ***n = 18***
Week 2	+1.4%	-1.1%

Mechanism of Action

Real World Data

Ongoing Investigations

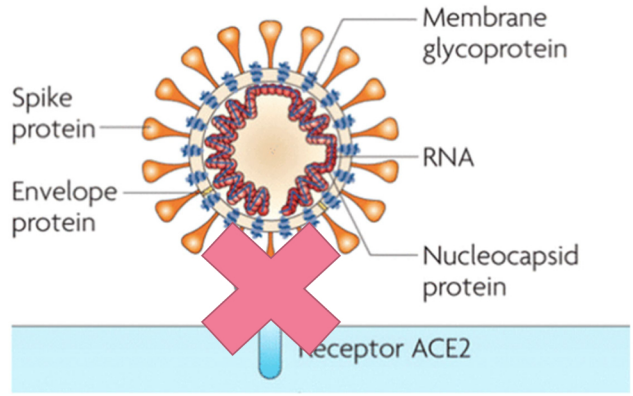
Humic Acid + Peptide

Mechanism of Action

Humic acid **encapsulates** virus and prevents binding to cell membrane receptors.

Peptide **binds to spike protein** and locks humic acid to target virus.

Humic acid peptide combination is indifferent to antigenic shift.



COVID-19: consider cytokine storm syndromes and immunosuppression

The Lancet, Published: March 16, 2020.
DOI: https://doi.org/10.1016/S0140-6736(20)30628-0

- A cytokine profile resembling secondary hemophagocytic lymphohistiocytosis (sHLH) is associated with COVID-19 disease severity, characterised by increased interleukin (IL)-2, IL-7, granulocyte-colony stimulating factor, interferon-γ inducible protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1-a, and **tumour necrosis factor-a.**[6]
- Predictors of fatality from a recent retrospective, multicentre study of 150 confirmed COVID-19 cases in Wuhan, China, included elevated ferritin (mean 1297·6 ng/ml in non-survivors *vs* 614·0 ng/ml in survivors; p<0·001) and IL-6 (p<0·0001),[2] suggesting that mortality might be due to virally driven hyperinflammation.

Valin A., et al., Crosstalk Between IL-6 and TNF-Alpha Signaling Pathway in Rheumatoid Arthritis Synovial Fibroblasts. American College of Rheumatology 2016 Annual Meeting

- **Almost 55% (287 genes) of total IL-6/sIL-6R regulated genes are common to those of TNF**-a.
- In addition, priming experiments stimulating fibroblast-like synoviocytes (FLS) cells with either TNF-a or IL-6/sIL-6R, followed by induction with the counterpart factor at dierent time-points, demonstrated that the synergistic eect requires the constant presence of both factors. These data suggest that **the mechanism of crosstalk between TNFa and IL-6/sIL-6R more likely occurs through regulation of signaling and/or transcriptional mediators** rather than at a post-transcriptional level. Our study suggests that despite the differential IL-6 and TNF-a intracellular signaling, **there is significant overlap between the transcriptional response induced by both factors.**

Real World Data (RWD)

As of February 2020: 285,100 doses of product were dispensed in China, Hong Kong, Singapore and South Korea.

RWD – Singapore, 2.2020

Family of 6 hospitalized, ages 20 – 54

Test positive for Covid-19 and transported to hospital by ambulance. Assay – 24-hour processing window (PCR)

- Treatment: 2 tablets or capsules three times day for 3 days
- Discharge criteria – negative assay x2, separated by 24–hours
  - First assay, 24-hours after initial treatment – negative
  - Second assay, 48 hours after initial treatment – negative
- All family members, discharged, hospital day #3 without complications

RWD, Hong Kong, March/April 2020

Background: Center Manager 1 (CM1) was exposed to a client with known COVID-19 infection at Distributor's business location. CM1 was taking Product and did not get sick, but a co-worker who was known not to be taking Product (CM2) and co-mingled with CM1 and client did become symptomatic with fever, chills and emesis. She ultimately tested positive for COVID-19 and was hospitalized.

RWD, Hong Kong

(2 of 3)

Hospital course: CM2's presenting complaints in hospital included fever, chills and emesis. Additional clinical information is forthcoming as of 4.3.2020 at 4:08PST. CM2 declined to take Product in hospital because her PTP was treating with another medication and she was concerned about an unintended interaction; however, symptoms were deteriorating. Distributor obtained consent from CM2 to start Product on 4.2.2020 at treatment dose. Twenty-four hours after completing an initial treatment dose, fever was resolved. As of 4.4.2020, CM2 is asymptomatic. F/U assay for COVID-19 is pending.

Note: Hong Kong practice is to publish the names of those infected with COVID-19 in the public record. CM2 information was published on 4.2.2020.

RWD, Hong Kong
(3 of 3)

Employer Response: Distributor became aware of CM2's infection on 3.31.2020 and closed local operation for 4 days. At this juncture, all employees were instructed to take a treatment dose of Product, 3 tablets three times daily. Distributor is performing PCR tests on all employees prior to their return to work. All employees are also part of a nightly webinar where they can learn about COVID-19 and ask questions about Product in order to earn compliance with Product prophylactic dosing.

Product Instructions

- Prophylactic directions: Take 1 tablet/capsule twice daily
- Prophylactic directions: Take 1 tablet/capsule 3 times daily
  - Chronic Disease - diabetic, heart disease
  - Autoimmune disease – rheumatoid arthritis, IBD, lupus
  - Overfat
  - Genomic Predisposition (NLRP3, CCL2, IL-1A)
- Treatment, COVID-19(+): Take 2 tablets/capsules by mouth three times daily
- Treatment, COVID-19(+) with morbidity: Take 3 tablets/capsules by mouth three times daily

In vitro study

In process as of 28.3.2020

- Title: ***In Vitro measurement of viral fusion inhibition and viricidal activity of CZV2.14 and CZV2.14+Humic Acid against SARS-CoV-2 in a human cell line with IC50 and IC90 benchmarks***

- Aim: *At the conclusion of this study, the in vitro potential of CZV2.14 and CZV2.14+Humic Acid to inhibit viral fusion and exert viricidal activity against SARS-CoV-2 in a human cell line will be quantified using a viral plaque assay, transmission electron microscopy, IC50 and IC90 measurements against multiple concentrations of CZV2.14 vs. CZV2.14+Humic Acid*

Toxicity Assays – Humic Acid

Table I. Toxic Concentrations at 50% (TC_{50}) of Humate Materials with Indicated Cell Lines

	TC_{50}, µg/mL					
				LLC-MK_2[d]		
Humate	BSC-1[a]	HFF[b]	MDCK[c]	Trial 1	Trial 2a[f]	Trial 2b[g]
CA	>100	>100	>100	>100	>1000	>1000
CGA	>100	>100	–[e]	–[e]	>1000	>1000
HA	>100	>100	>100	>100	>1000	>1000
HGA	>100	>100	–[e]	–[e]	700	>1000

[a] African green monkey kidney cells. [b] Human foreskin fibroblast cells. [c] Madin Darby canine kidney cells. [d] Adult rhesus monkey kidney cells. [e] Not evaluated. [f] Neutral Red assay. [g] Visual assay.

Not cytotoxic at levels as least as high as 100ug/mL.

Broad Spectrum Antiviral Effectiveness of Natural and Synthetic Humates; Virology Branch, Antiviral Research & Antimicrobial Chemistry Program, Division of Microbiology & Infectious Diseases; Screening & Testing Program for Antiviral, Immunomodulatory, Anti-tumor and/or Drug Delivery Activities, National Institutes of Allergy & Infectious Diseases, National Institute of Health (NIH), August 9, 2002

Toxicity Assays
Humic Acid

- Up to 50mg/kg

- Case example: 75kg male
  - 3750mg/day
  - Actual maximum dose, HA = 2250mg/day in current product iteration

Schiller F, Klocking R, Wutzler P, Farber I. Results of an oriented clinical trial of ammonium humate for the local treatment of herpesvirus hominis (HVH) infections. Dermatol Monatsschr. 1979 Jul; 165(7): 505-9

Toxicity Assays

Humic Acid + Peptide

Additional studies are not warranted at this time given the current ratio of humic acid : peptide = 250mg : 5ug per tablet or capsule

Schiller F, Klocking R, Wutzler P, Farber I. Results of an oriented clinical trial of ammonium humate for the local treatment of herpesvirus hominis (HVH) infections. Dermatol Monatsschr. 1979 Jul; 165(7): 505-9

Cell Proliferation Study
Humic Acid

All humates except for synthetic CA with Daudi cells did not inhibit cell proliferation at drug levels at least as high as 50 μg/mL, as shown below in **Table II.**

Table II. Cell Proliferation Inhibition Concentrations at 50% (CP_{50}) of Humate Materials with Indicated Cell Lines

	CP_{50}, μg/mL	
Humate	HFF[a]	Daudi[b]
CA	71.2	<0.08
CGA	96	>50
HA	88.4	>50
HGA	>100	>50

[a] Human foreskin fibroblast cells. [b] Burkitt's lymphoma derived cells.

Broad Spectrum Antiviral Effectiveness of Natural and Synthetic Humates; Virology Branch, Antiviral Research & Antimicrobial Chemistry Program, Division of Microbiology & Infectious Diseases; Screening & Testing Program for Antiviral, Immunomodulatory, Anti-tumor and/or Drug Delivery Activities, National Institutes of Allergy & Infectious Diseases, National Institute of Health (NIH), August 9, 2002

Frequent talking points, 2020.0402

- Attack the body
  - Stimulates a cytokine storm
    - Chronic disease
    - Genomics – NLRP3 and CCL2; IL-1B, CARD8, TNF-a, IL-6
- How infectious:
  - Greater than SARS 2002-2004 (10-20x greater) and influenza.
  - Less than measles and tuberculosis
  - Half life – aerosol 1.2 hrs, on plastic 6.8 hrs, on stainless steel 5.6 hrs, BUT STILL VIABLE AFTER 72 HOURS on some surfaces
  - Serious discussion in US of wearing mask if NOT infected. Studies in China showed transmission > 6 feet in hospital settings