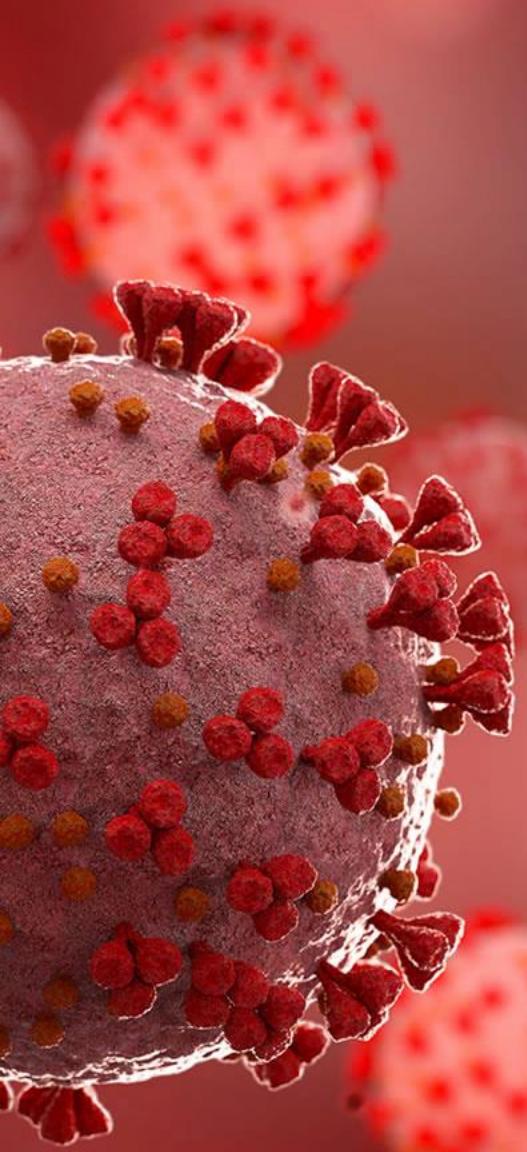


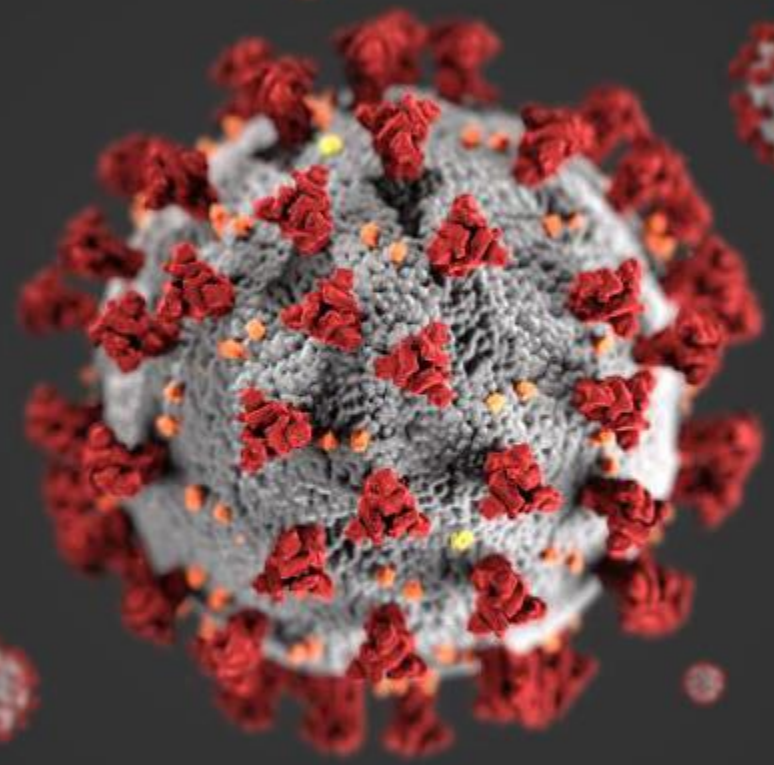
Wise Traditions

IN SILICO GENOMICS:
THE NEW PARADIGM OF VIRUS SIMULATION
-AND-
PATHOGENIC PRIMING:
WHAT DOES THE SCIENCE ACTUALLY SHOW?

Andrew Kaufman, MD

FACT No. 1

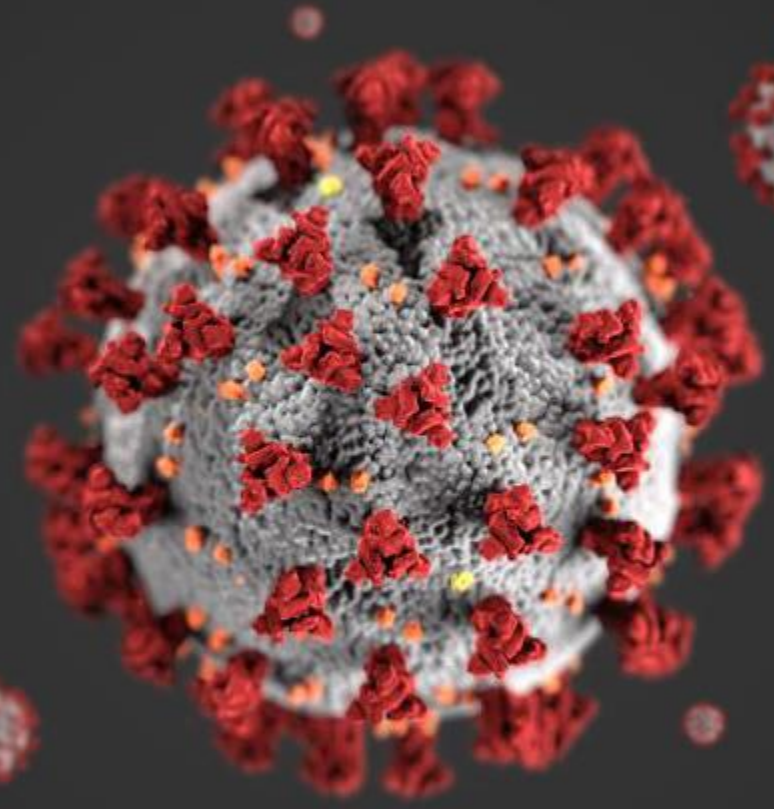
SARS-CoV-2 Does NOT EXIST!!



Computer generated, 3D Biomedical Art
by artists Alissa Eckert, and Dan Higgins
To Illustrate ultrastructural morphology
alleged to be exhibited by coronaviruses.

FACT No. 2

Covid-19 Does NOT EXIST!!



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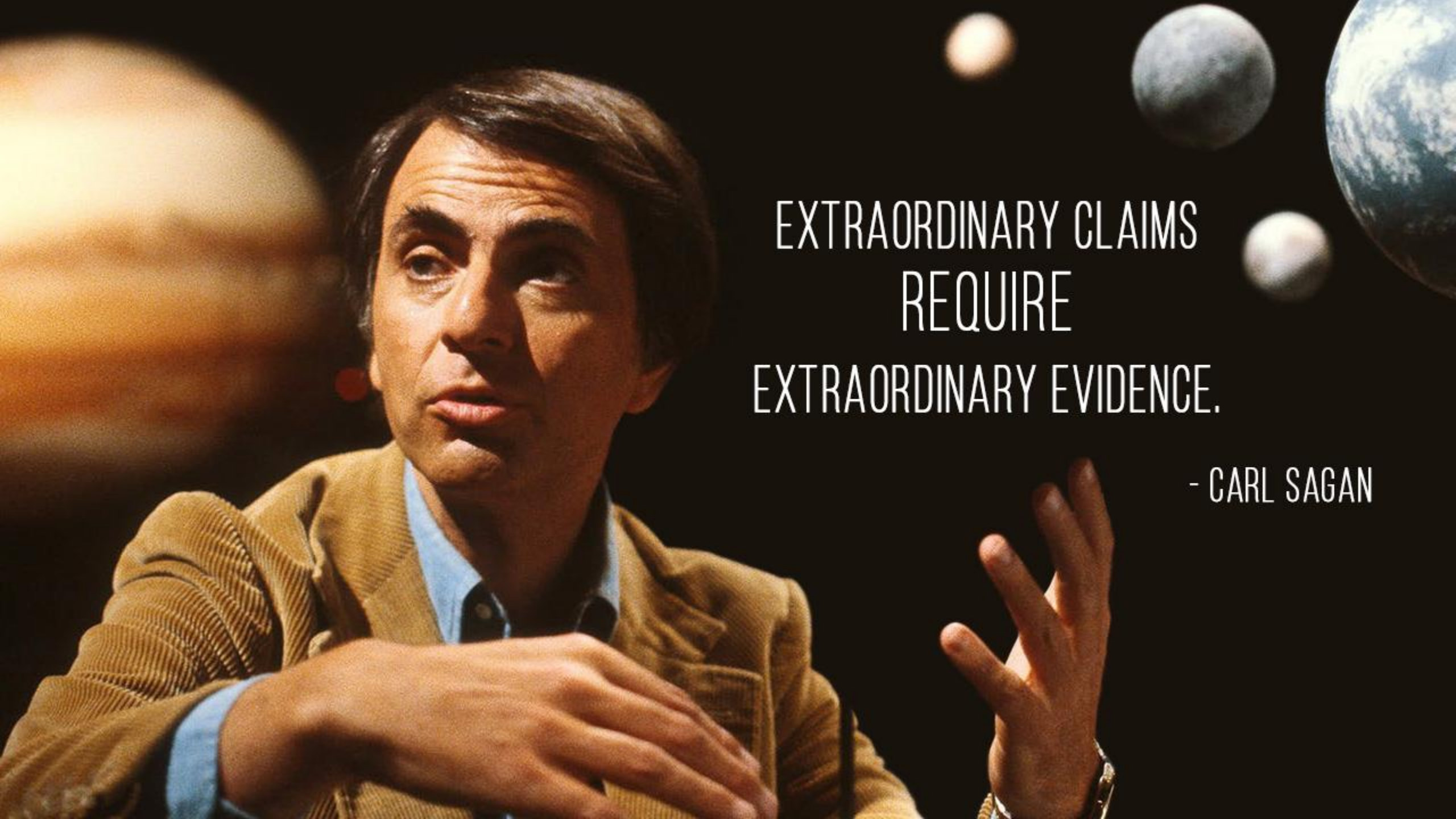

EXTRAORDINARY CLAIMS
REQUIRE
EXTRAORDINARY EVIDENCE.
- CARL SAGAN

The Extraordinary Claim:

A microscopic particle, which has never been seen, emerged out of nowhere, spread around the world, and caused the worst disease in over 100 years!

WHAT CAN BE ASSERTED WITHOUT EVIDENCE...

CAN BE DISMISSED WITHOUT EVIDENCE.

HITCHENS' RAZOR

Logical Fallacies

Informal

An **informal fallacy** refers to an argument whose proposed conclusion is not supported by the premises. This creates an unpersuasive or unsatisfying conclusion.

Ad Hominem*	Ambiguity*
Anecdotal*	Appeal to Authority*
Appeal to Emotion*	Appeal to Nature*
Appeal to Ridicule	Appeal to Tradition
Argument from Repetition	Argumentum ad Populum
Bandwagon*	Begging the Question*
Burden of Proof*	Circular Reasoning*
Continuum Fallacy	Equivocation*
Etymological Fallacy*	Fallacy Fallacy*
Fallacy of Composition and Division*	Fallacy of Quoting Out of Context
False Cause & False Attribution*	False Dilemma*

Logical Fallacies

Ad Hominem

The ad hominem attack is a logical fallacy associated with trying to undermine the opponent's arguments by personal attacks, through attacking their character or skill level, etc. The ad hominem attack uses an accepted fact about a person to undermine their credibility despite the lack of causal connection between the two parts of the argument.

Example of Ad Hominem

- Bill claims that this was an accident, but we know Bill to be a liar, so we can't take his word for it.

  Even though Bill may be a liar, his character does not automatically make anything he says untrue.

- Susan is an avid hunter, therefor she cannot possibly support gun control.

  Being a hunter is used as a negative characteristic to make a conclusion which could very well be untrue. Susan could support a variety of gun control elgislation.

“It can be proven that most claimed research findings are false.”

Essay

Why Most Published Research Findings Are False

John P. A. Ioannidis

Summary

There is increasing concern that most current published research findings are false. The probability that a research claim is true may depend on study power and bias, the number of other studies on the same question, and, importantly, the ratio of true to no relationships among the relationships probed in each scientific field. In this framework, a research finding is less likely to be true when the studies conducted in a field are smaller; when effect sizes are smaller; when there is a greater number and lesser preselection of tested relationships; where there is

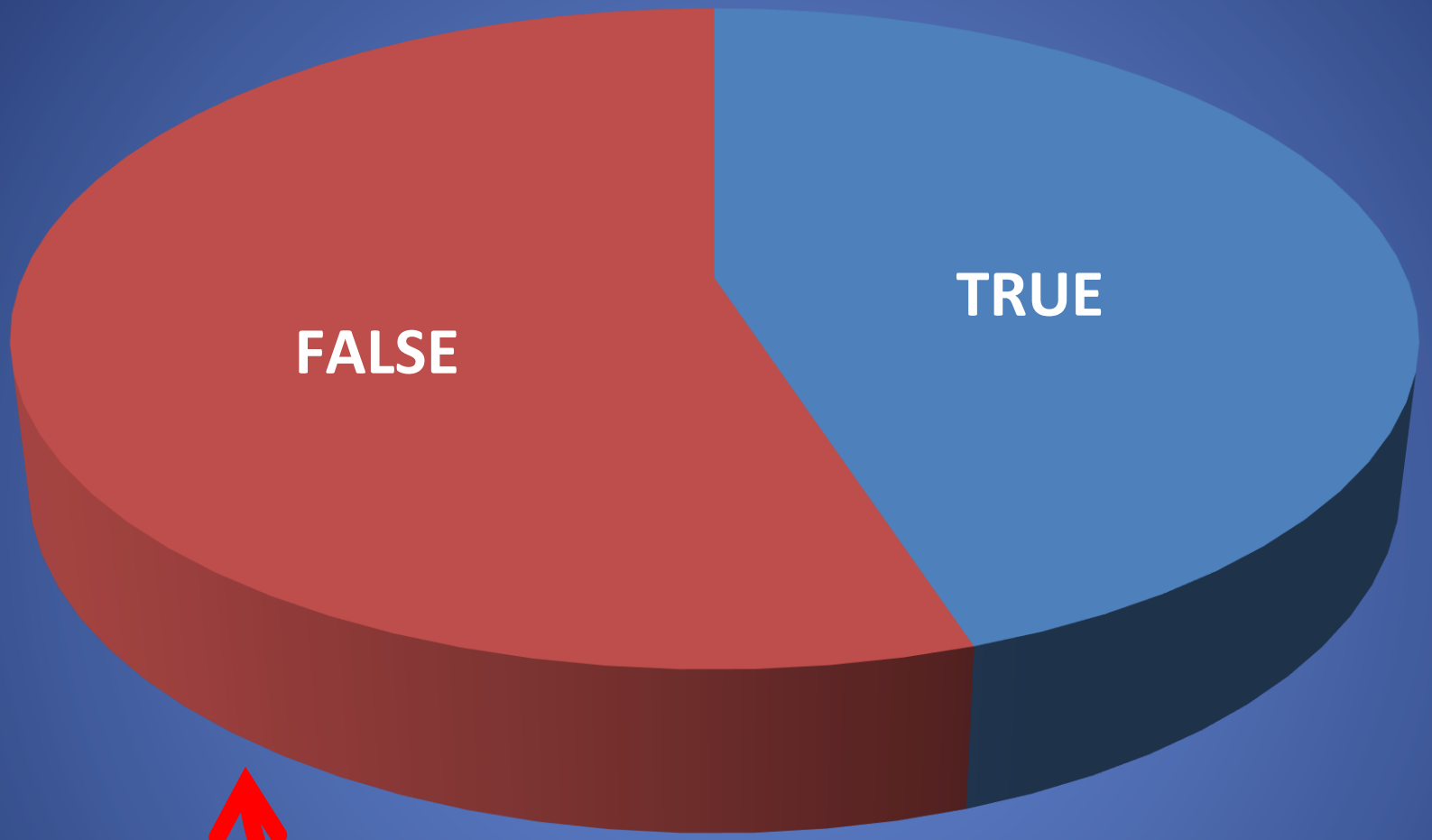
factors that influence this problem and some corollaries thereof.

Modeling the Framework for False Positive Findings

Several methodologists have pointed out [9–11] that the high rate of nonreplication (lack of confirmation) of research discoveries is a consequence of the convenient, yet ill-founded strategy of claiming conclusive research findings solely on the basis of a single study assessed by formal statistical significance, typically for a *p*-value less than 0.05. Research is not most appropriately represented

is characteristic of the field and can vary a lot depending on whether the field targets highly likely relationships or searches for only one or a few true relationships among thousands and millions of hypotheses that may be postulated. Let us also consider, for computational simplicity, circumscribed fields where either there is only one true relationship (among many that can be hypothesized) or the power is similar to find any of the several existing true relationships. The pre-study probability of a relationship being true is $R/(R + 1)$. The probability of a study finding a true relationship

Research Findings



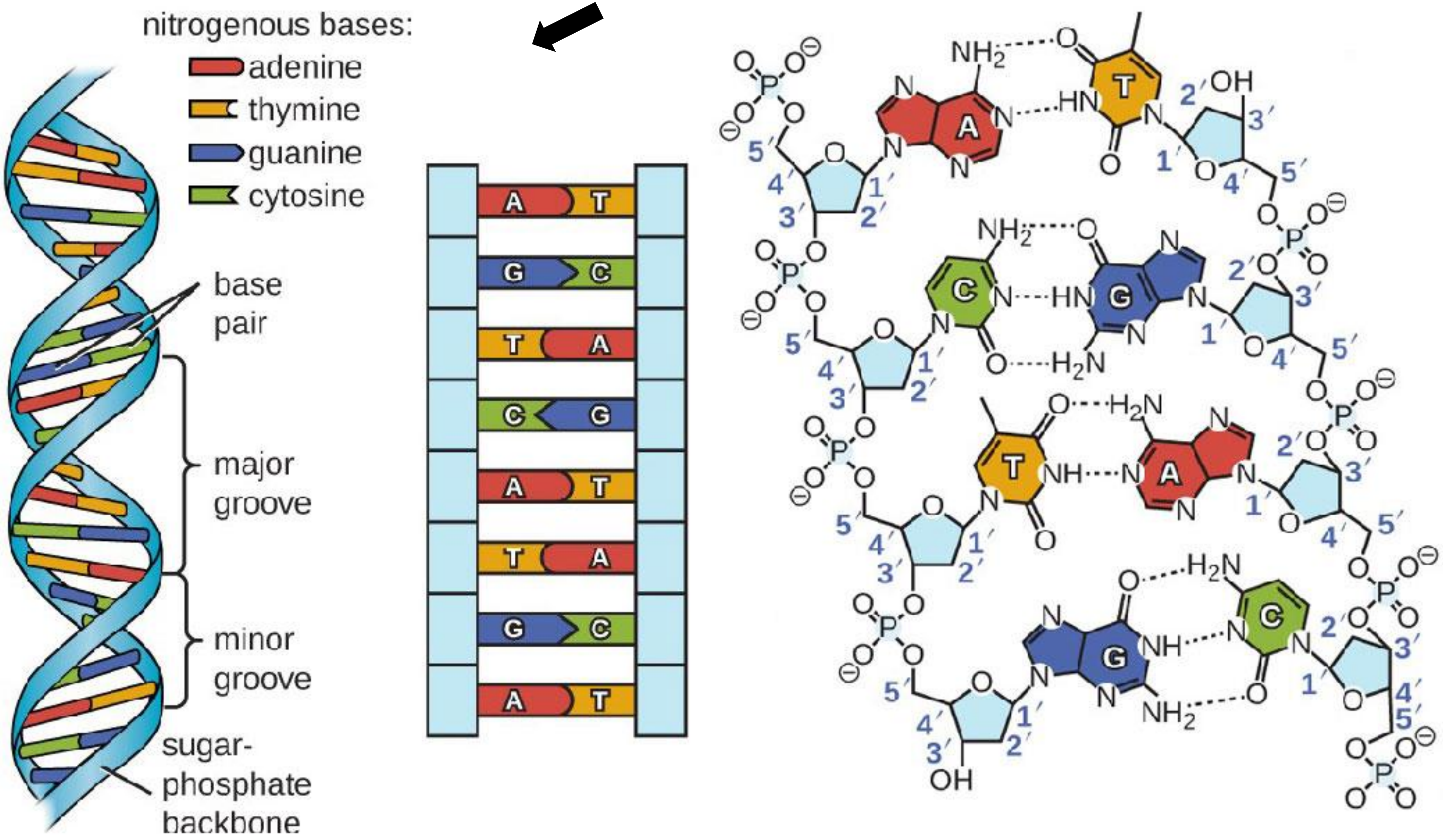
Virology Research

THE ‘IN SILICO’ GENOME

AND

THE NEW PARADIGM OF GENETIC VIROLOGY

WHAT IS DNA?

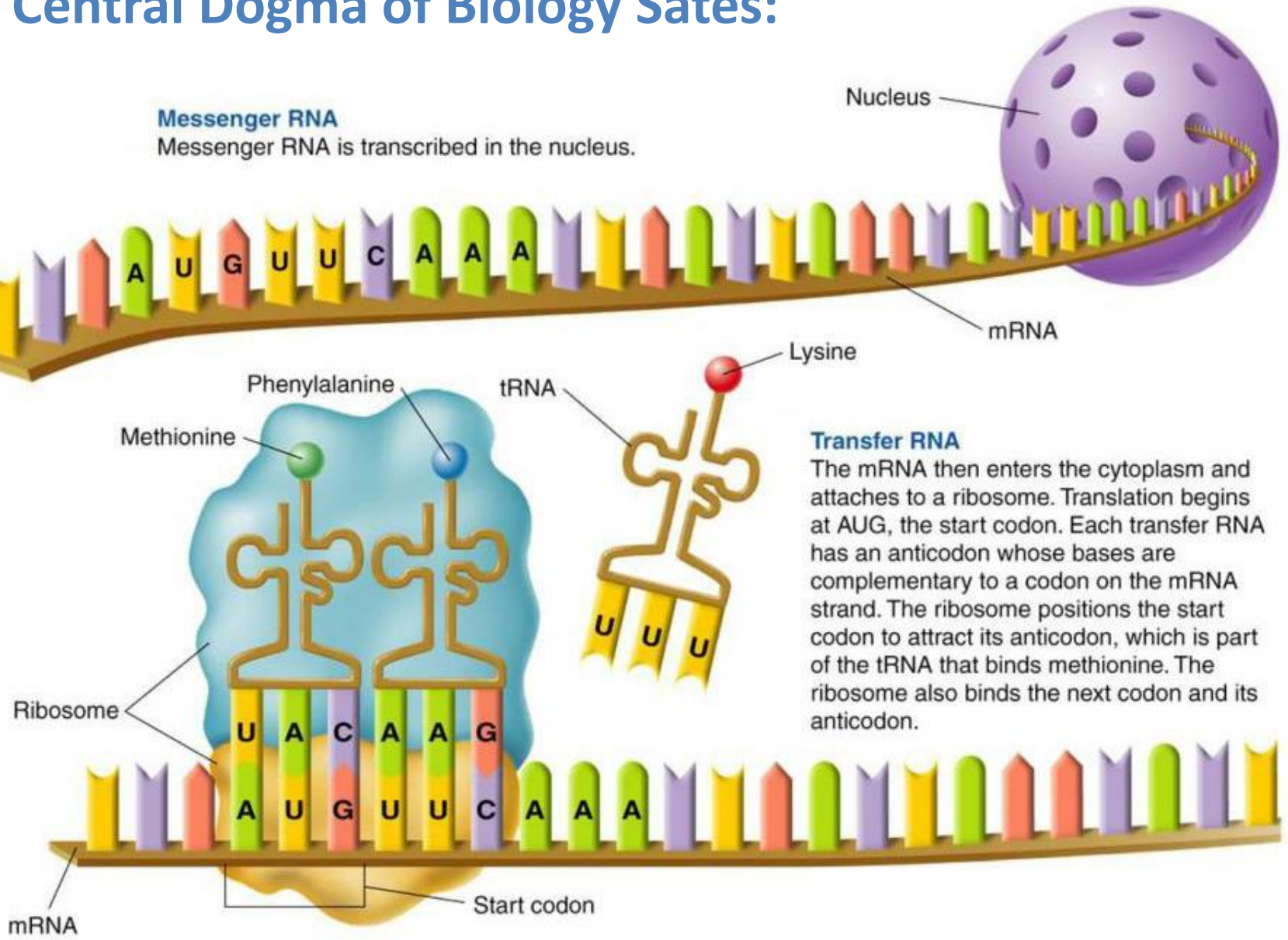

nitrogenous bases:
adenine
thymine
guanine
cytosine
base pair
major groove
minor groove
sugar-phosphate backbone
A T
G C
T A
C G
A T
T A
G C
A T

WHAT IS DNA?

The Central Dogma of Biology Sates:

DNA into RNA into Proteins

Each of the three letters code for an amino acid (the building blocks of proteins)

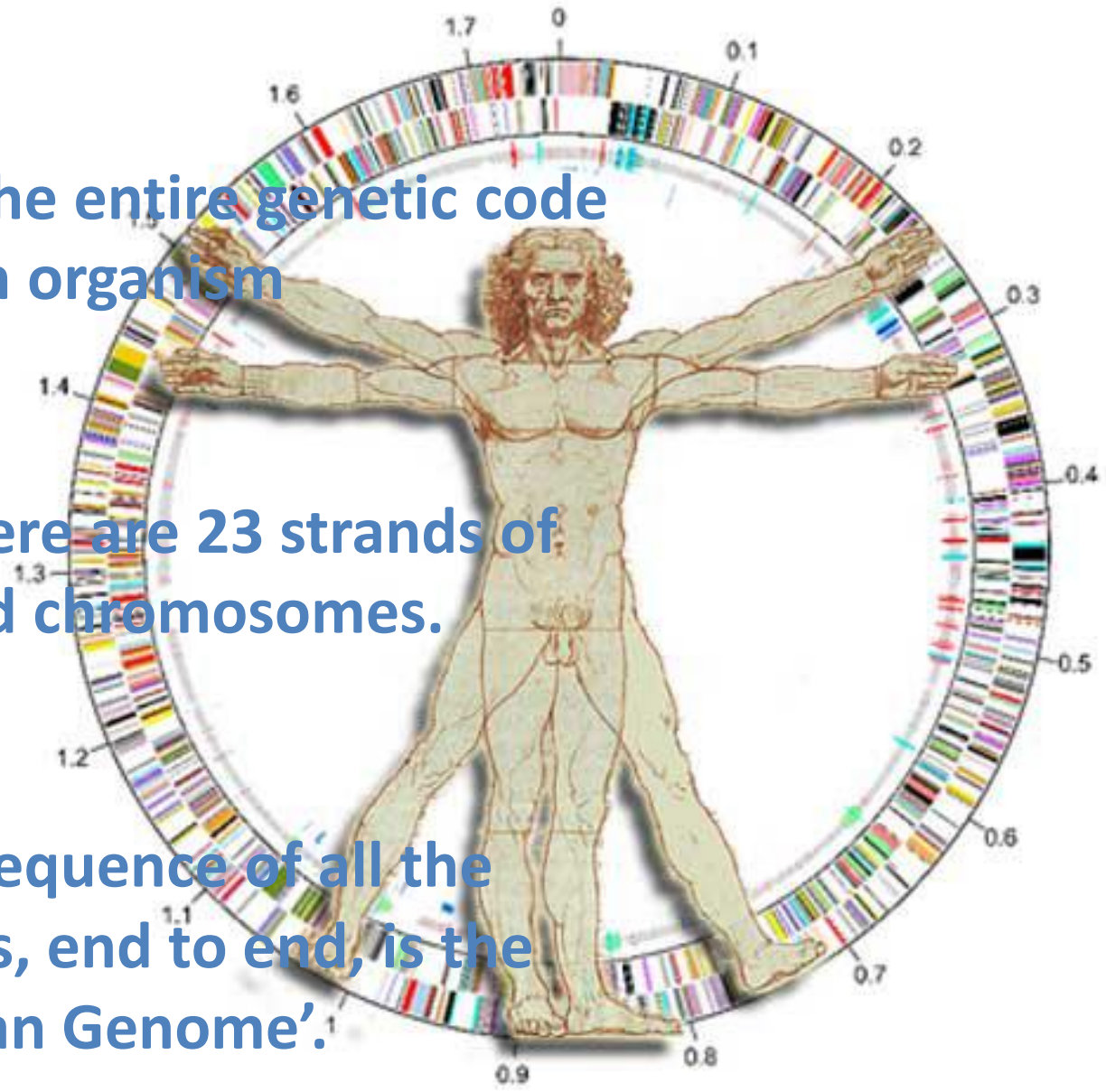


WHAT IS THE GENOME?

The genome is the entire genetic code of an organism

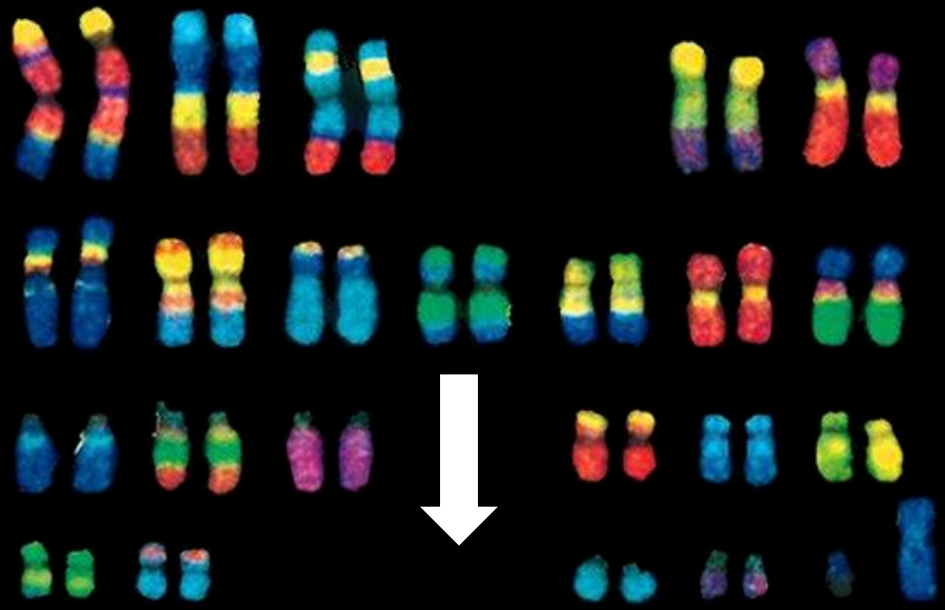
In humans, there are 23 strands of DNA called chromosomes.

The entire sequence of all the chromosomes, end to end, is the ‘Human Genome’.



How To Sequence A Genome:

Actual Human
(Non-Theoretical)



HUMAN CHROMOSOMES

GTACGTCCAATGCGTACGTAGGTTATCGAGCTGAGGTTAGGCTTAGGCGCGT
TAGAAATCGGATCGGATCGATCGATCGTGACACGTACTGTTGGACCTGACCC
AATGTTCATGGACTTTGCGTGCATGGGTGGGCGCATAGCGTAGAAGGTTCC
CGTAGTGTTGCGTACTTAGAAATCGGATCGGTACTAGGTTATCGATCGGATC
GGATCCCAATGCAGCGTAGAAGGTTTCGTGACACGTACTGTTGGACCTGAC
CCAATGTGACCCAATGTTCATGGACTTTGCGTGCATGGGTGGGCGCATAGCG
TAGAAGGTTCCCGTAGTGTTGCGTACTTAGAAATCGGATCGGTACTAGGTTA
TCGATCGGATCGGATCCCAATGCAGCGTAGAAGGTTTCGTGACACGTACTGT
TGGACCTGACTTCCCGTAGTGTTGCGTACTTGGTTATCGAGCTGAGGTTAGG
CTTAGGCGCGTTAGAAATCGGATCGGATCGATCGATCGTGACACGTACTGTT
GGACCTGACCCAATGTTCATGGACTTTGCGTGTACGTCCAATGCGTACGTAG
GTTATCGAGCTGAGGTTAGGCTTAGGCGCGTTAGGCAAATCGGATCGGATC
G A T C G A T C G T G A C A C G T A C T G T T G G A C C T G A C C C

Is this how they sequence the genomes of viruses?

NO SIR!

How to sequence An ‘in silco’ Genome

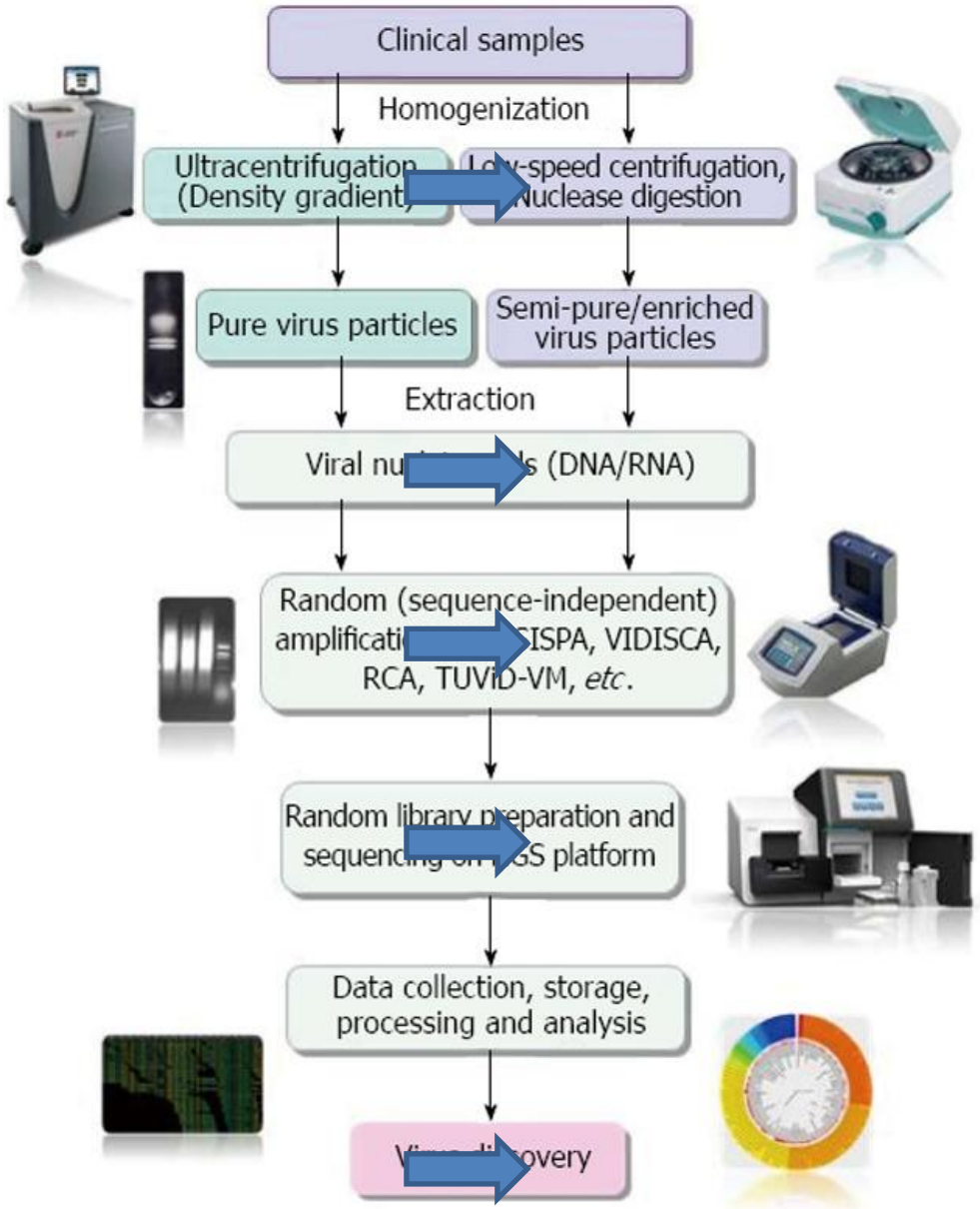
In silico (adjective)

literally ‘in silicon’, i.e. ‘in the computer’; referring to analysis or experimentation carried out in a computer environment, rather than in the laboratory. It is the mimicking or modeling of biological processes within computer hardware and software.

In other words:

IMAGINARY

“NEXT GENERATION” SEQUENCING



- **This purification step is NOT DONE with SARS-CoV-2**
- **The source of the RNA is UNKNOWN**
- **Amplifies EVERYTHING in the sample**
- **Alignment**
- **Discovery of a “Theoretical Virus”**

FIRST AND ORIGINAL SARS-CoV-2 GENOME REPORTED

Article

A new coronavirus associated with human respiratory disease in China

https://doi.org/10.1038/s41586-020-2008-3
Received: 7 January 2020
Accepted: 28 January 2020
Published online: 3 February 2020
Open access
Check for updates

Fan Wu[1,7], Su Zhao[2,7], Bin Yu[3,7], Yan-Mei Chen[1,7], Wen Wang[4,7], Zhi-Gang Song[1,7], Yi Hu[2,7], Zhao-Wu Tao[2], Jun-Hua Tian[3], Yuan-Yuan Pei[1], Ming-Li Yuan[2], Yu-Ling Zhang[1], Fa-Hui Dai[1], Yi Liu[1], Qi-Min Wang[1], Jiao-Jiao Zheng[1], Lin Xu[1], Edward C. Holmes[1,5] & Yong-Zhen Zhang[1,4,6]✉

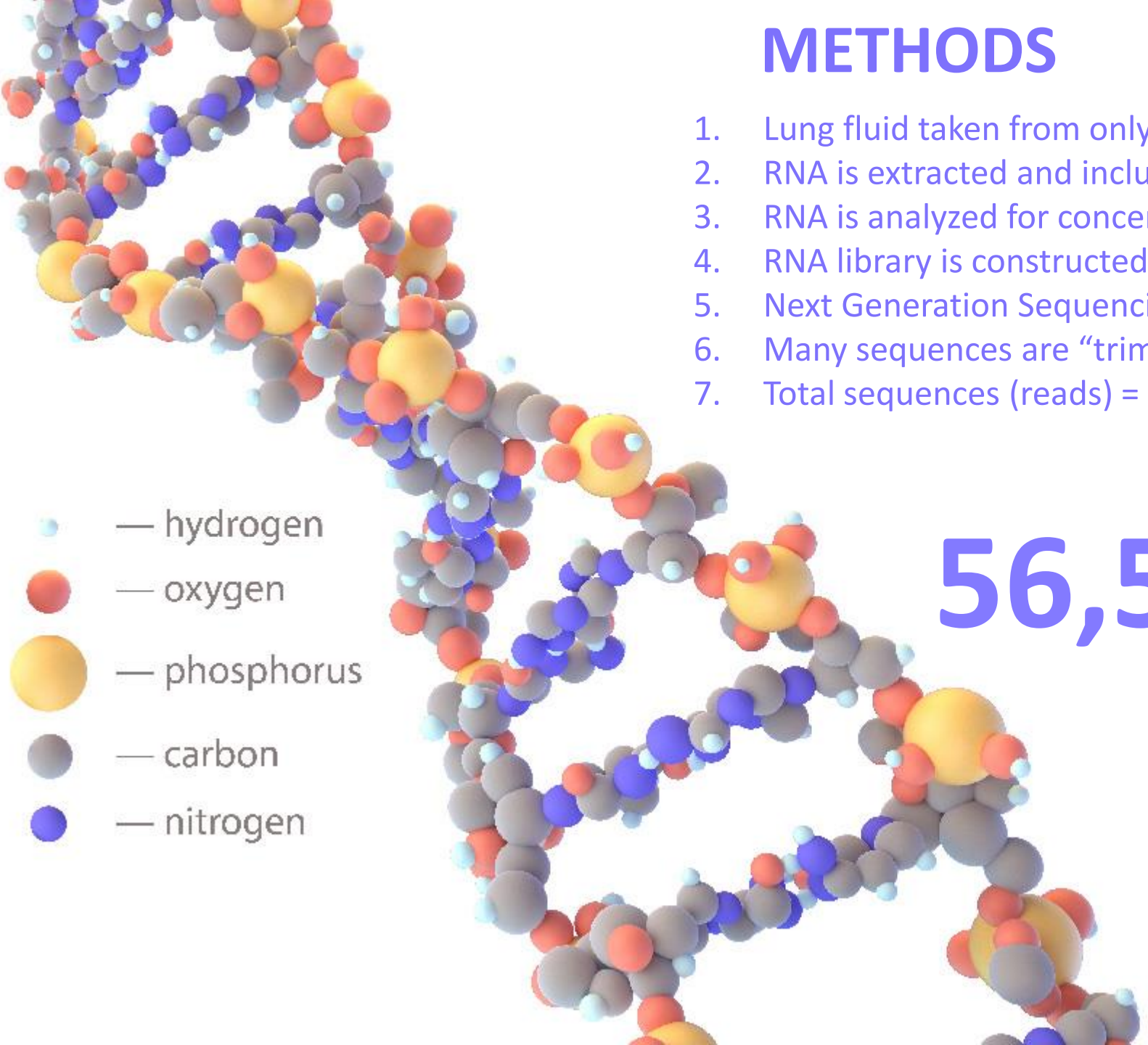
Emerging infectious diseases, such as severe acute respiratory syndrome (SARS) and Zika virus disease, present a major threat to public health[1–3]. Despite intense research efforts, how, when and where new diseases appear are still a source of considerable uncertainty. A severe respiratory disease was recently reported in Wuhan, Hubei province, China. As of 25 January 2020, at least 1,975 cases had been reported since the first patient was hospitalized on 12 December 2019. Epidemiological investigations have suggested that the outbreak was associated with a seafood market in Wuhan. Here we study a single patient who was a worker at the market and who was admitted

[1]Shanghai Public Health Clinical Center, Fudan University, Shanghai, China. [2]Department of Pulmonary and Critical Care Medicine, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. [3]Wuhan Center for Disease Control and Prevention, Wuhan, China. [4]Department of Zoonosis, National Institute for Communicable Disease Control and Prevention, China Center for Disease Control and Prevention, Beijing, China. [5]Marie Bashir Institute for Infectious Diseases and Biosecurity, School of Life and Environmental Sciences and School of Medical Sciences, The University of Sydney, Sydney, New South Wales, Australia. [6]School of Public Health, Fudan University, Shanghai, China. [7]These authors contributed equally: Fan Wu, Su Zhao, Bin Yu, Yan-Mei Chen, Wen Wang, Zhi-Gang Song, Yi Hu. ✉e-mail: zhangyongzhen@shphc.org.cn

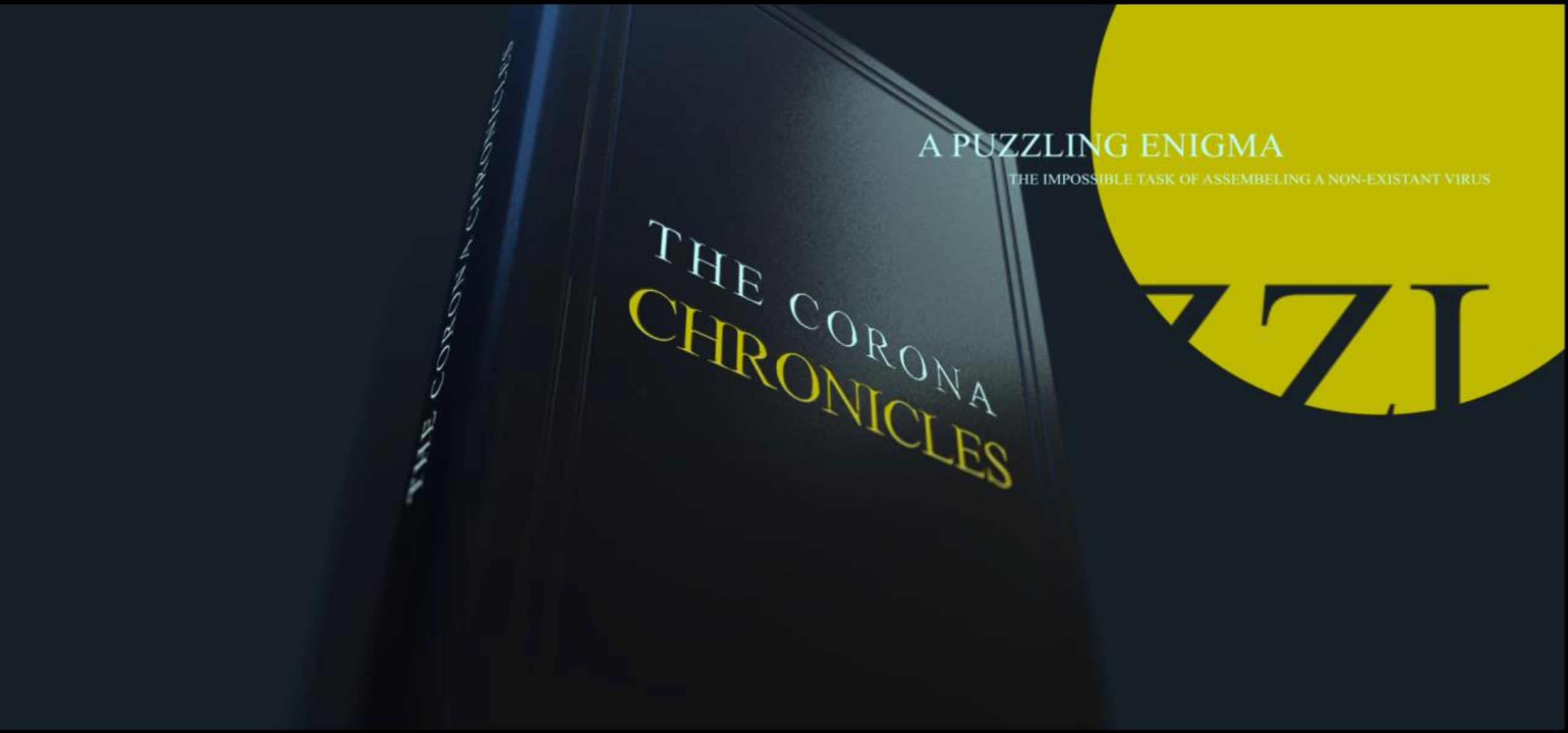
METHODS

1. Lung fluid taken from only ONE “suspected case”
2. RNA is extracted and includes all sources of RNA (human, microbial)
3. RNA is analyzed for concentration and length
4. RNA library is constructed
5. Next Generation Sequencing. Only short strands used! (150 bases)
6. Many sequences are “trimmed”
7. Total sequences (reads) =

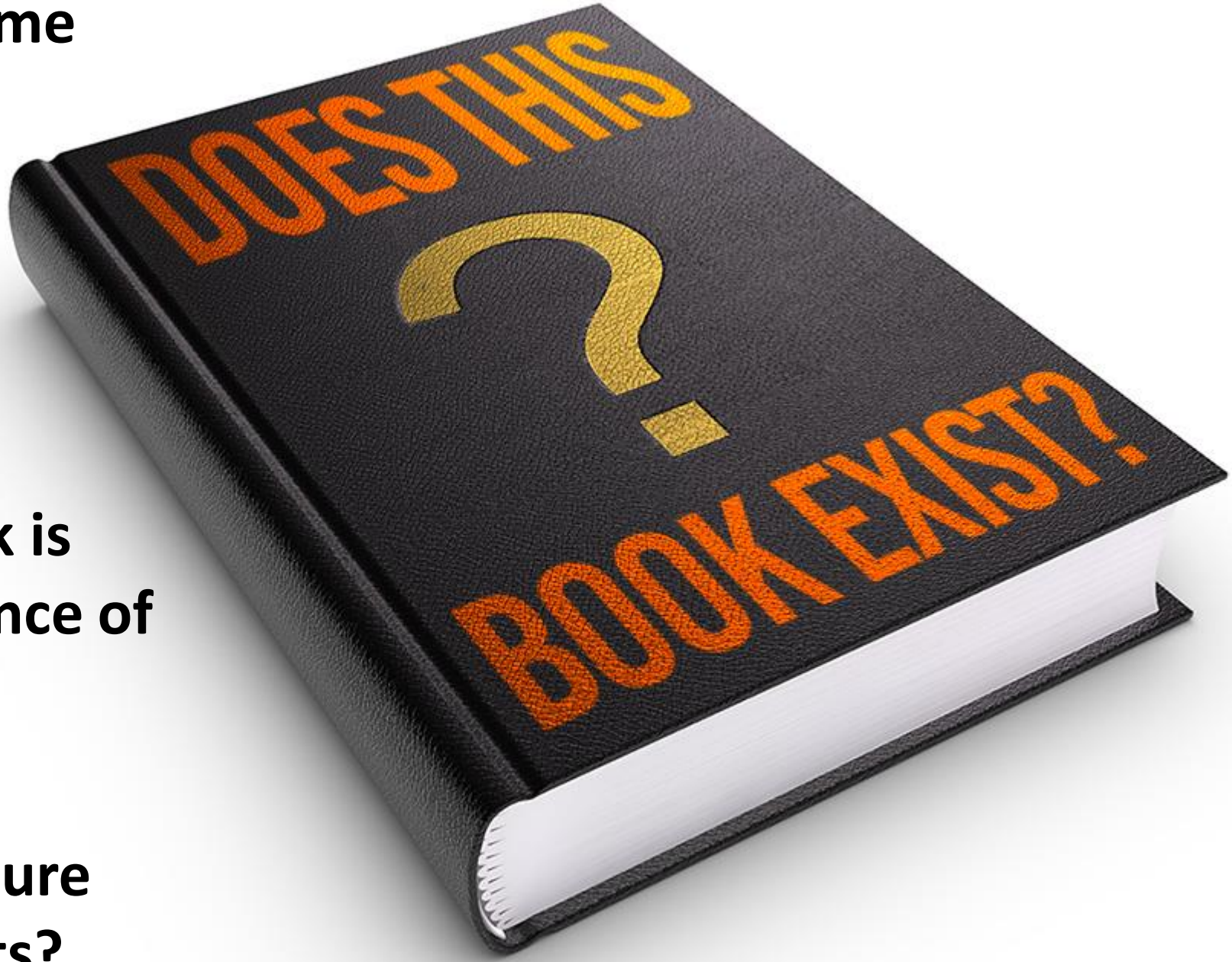
56,565,928



HOW
DO THE

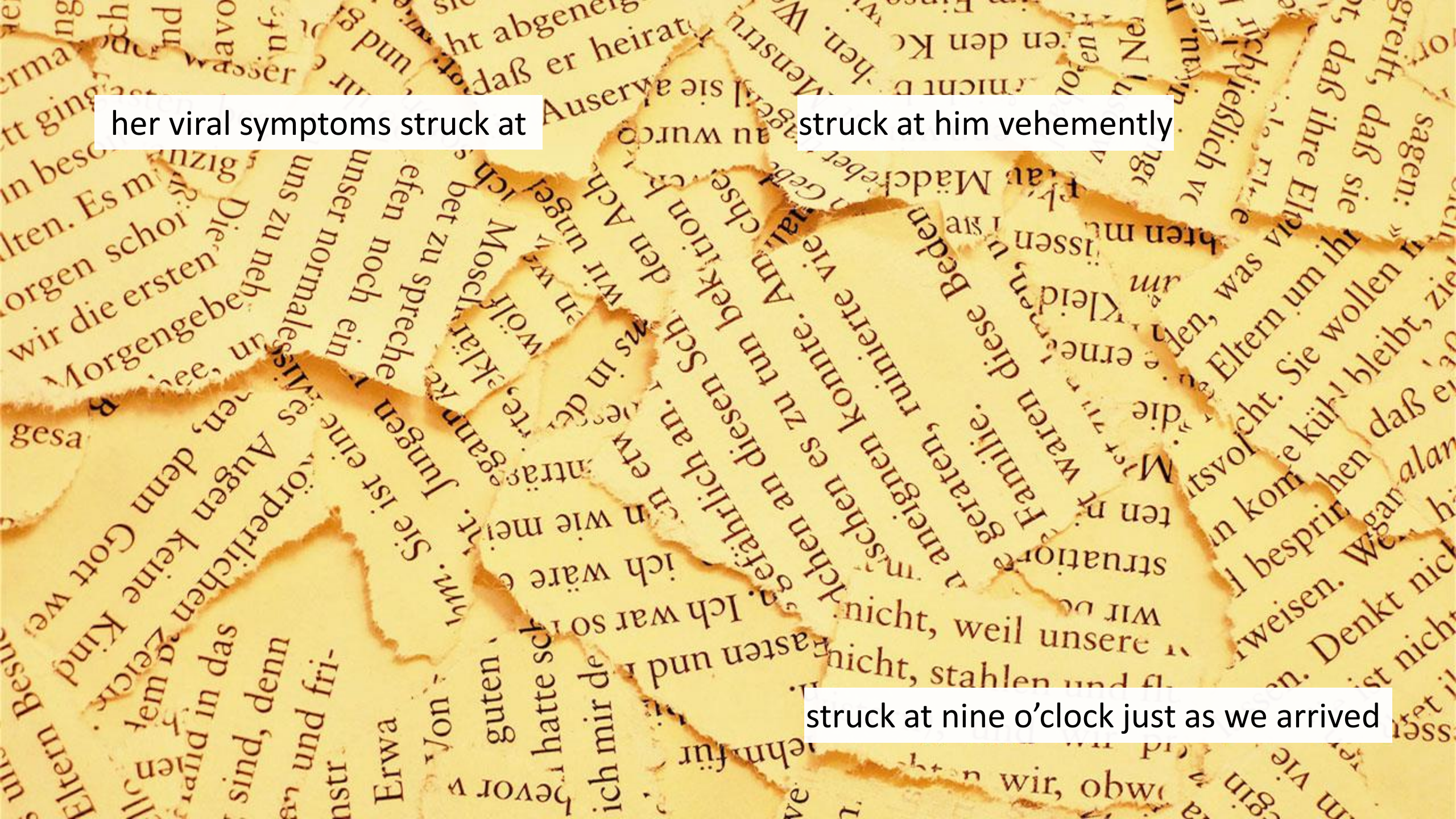

THE CORONA CHRONICLES
A PUZZLING ENIGMA
THE IMPOSSIBLE TASK OF ASSEMBELING A NON-EXISTANT VIRUS
THE CORONA CHRONICLES

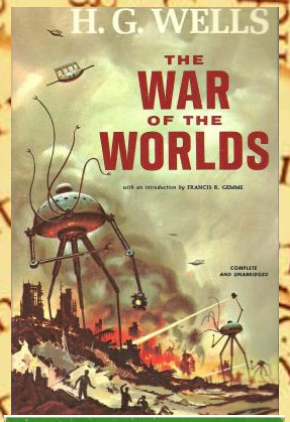
Sequencing a viral genome is like reading a book.

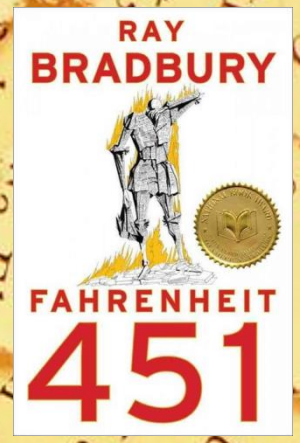


The genome of the book is simply the entire sequence of letters.

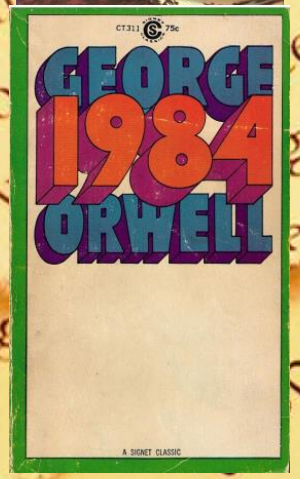
But, what if you aren't sure that the book even exists?


her viral symptoms struck at
struck at him vehemently
struck at nine o’clock just as we arrived

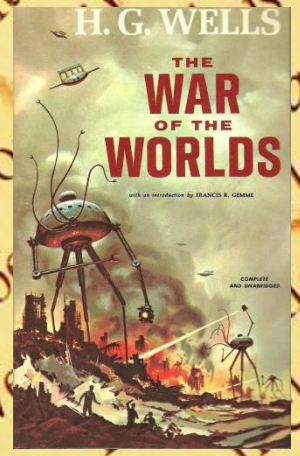




I was at home at that hour and **writing in** kindergarten and first grade.



The teachers of our country have to be taught to start teaching reading and **writing in** a foolish facetious tone that the perfection of mechanical appliances must ultimately supersede limbs

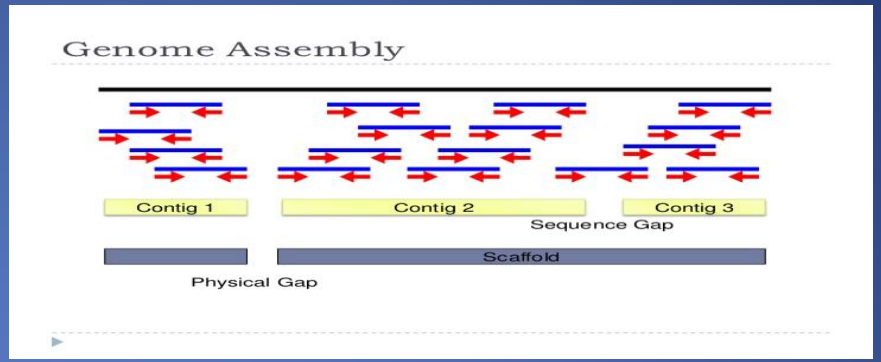


For a moment he was seized by a kind of hysteria. He began **writing in** my study, and although my French windows face towards Ottershaw and the blind was up (for I loved in those days to look up at the night sky), I saw nothing of it.

ASSEMBLED ‘CONTIG’

at that hour, in the teachers of our foolish facetious tone that the perfection superseded limbs seized by a kind of hysteria. He began writing although my French windows faced towards victimization and the blind in those days look up at the night sky. I saw nothing of puffed mechanical appliances? The body of mind doesn't like afternoon papers ultimately clashing against big headlines of taxing rhapsodic. A wave, loudly set a treehouse on fire! What with the future yet to come, smirkingly prodigious profile lies the guttural reward a head. Whiskey needs a shower in the morning dew of wondrous awe where the world racked by a trees utter nonsensical love. The other side sat down once more and passionate serendipity slips on a caring mother yet still not coherent where our

ASSEMBLY OF CONTIGS

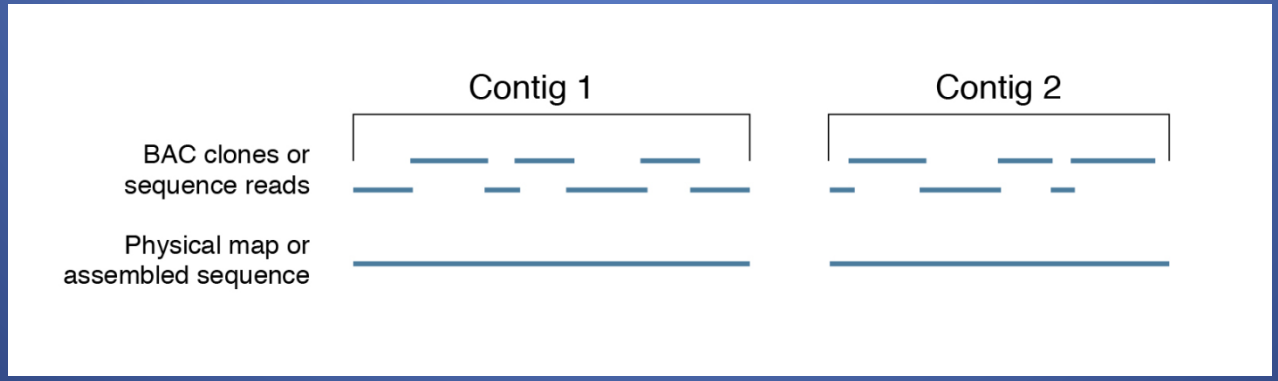


Megahit	Trinity
384,096 Contigs	1,329,960 Contigs
Range 200 – 30,474	Range 201 – 11,760

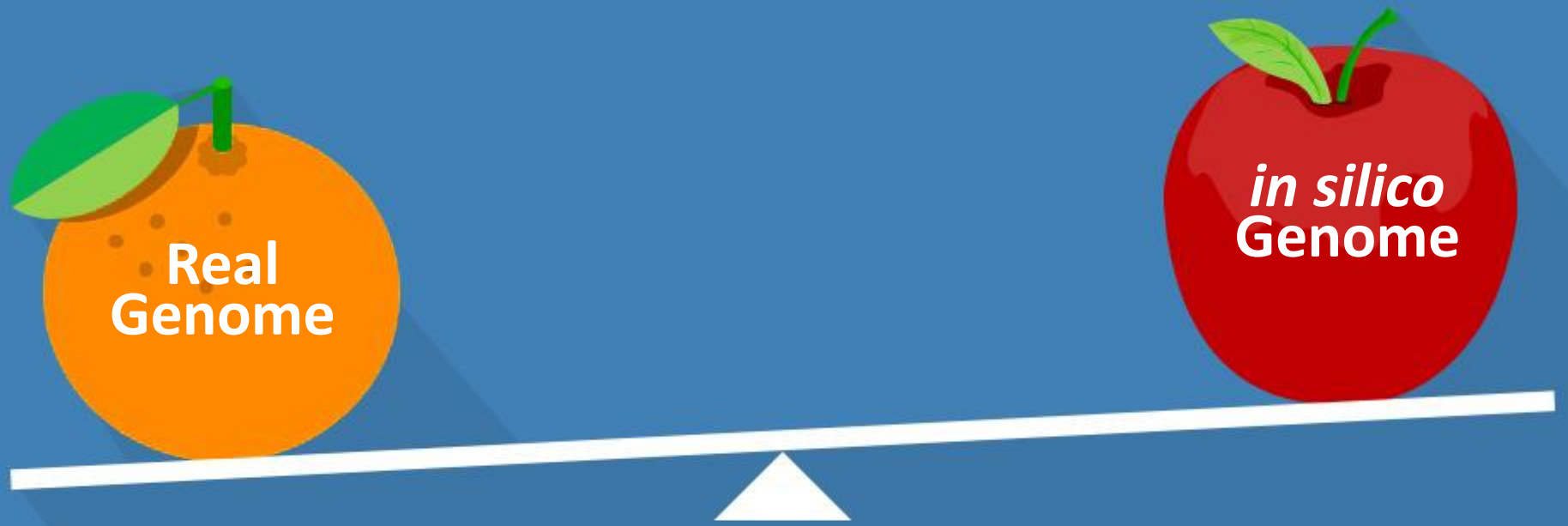
- Longest Contig (30,474) Chosen!
  - 89.1% Similar to bat SARS-like CV isolate sequence
- Claimed whole genome 29,903 bases long
  - What happened to the other 571 bases?
  - Assembled from 123,613 reads

ASSEMBLY OF CONTIGS

- How did they know which reads were correct?
- How do they know this genome is real?
- How do they know the sequences' origin?
- How much error is there in this process?
- Can the results be replicated?



COMPARE AND CONTRAST



Comes from:
Intact, isolated, and characterized organism

Can be reproduced without error

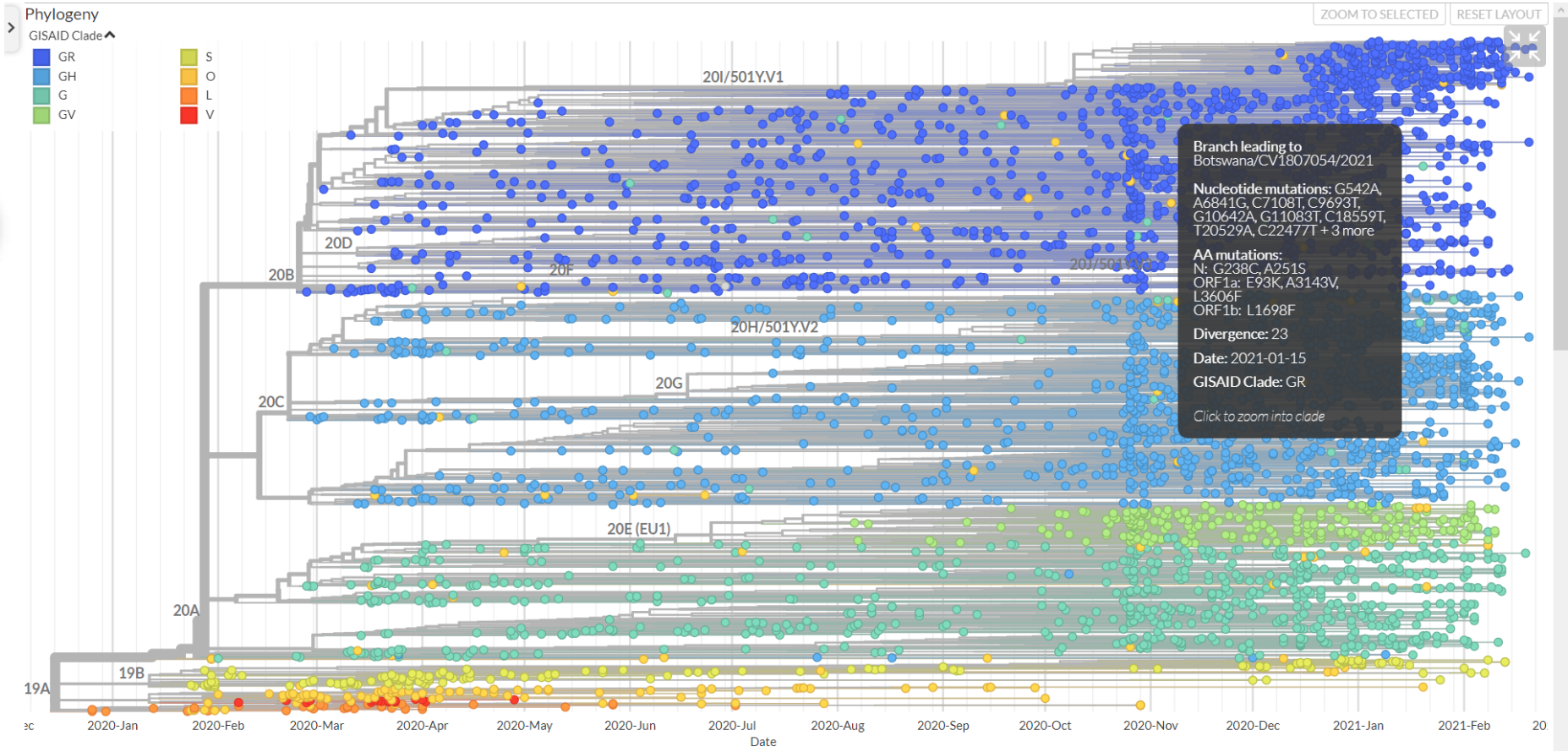
Represents:
ACTUAL code, an ACTUAL organism

Comes from:
A mixture of unknown sources

Cannot reproduce without error, false mutations

Represents:
THEORETICAL code, a THEORETICAL organism

VARIANTS
WHAT ARE THEY?



Science & Research

Science Agenda for COVID-19

Weekly Review +

Science Briefs +

COVID Data Tracker

Forecasting +

Variant Surveillance −

Genomic Surveillance for SARS-CoV-2

Tracking Emerging Variants

SARS-CoV-2 Variant Classifications

SARS-CoV-2 Variant Classifications and Definitions

Updated Aug. 3, 2021 Print

Key Points

- Genetic variants of SARS-CoV-2 have been emerging and circulating around the world throughout the COVID-19 pandemic.
- Viral mutations and variants in the United States are routinely monitored through sequence-based surveillance, laboratory studies, and epidemiological investigations.
- A US government SARS-CoV-2 Interagency Group (SIG) developed a Variant Classification scheme that defines three classes of SARS-CoV-2 variants:
  - Variant of Interest
  - Variant of Concern
  - Variant of High Consequence

Get Variant Classification and Definition Updates

To receive email updates when a variant classification or definition changes, enter your email address:

Email Address

What's this? Submit

Cold Spring Harbor Laboratory

bioRxiv

THE PREPRINT SERVER FOR BIOLOGY

bioRxiv posts many COVID19-related papers. A reminder: they have not been formally peer-reviewed and should not guide health-related behavior or be reported in the press as conclusive.

New Results

Antibody Resistance of SARS-CoV-2 Variants B.1.351 and B.1.1.7

Pengfei Wang, Manoj S. Nair, Lihong Liu, Sho Iketani, Yang Luo, Yicheng Guo, Maple Wang, Jian Yu, Baoshan Zhang, Peter D. Kwong, Barney S. Graham, John R. Mascola, Jennifer Y. Chang, Michael T. Yin, Magdalena Sobieszczyk, Christos A. Kyratsous, Lawrence Shapiro, Zizhang Sheng, Yaoxing Huang, David D. Ho

doi: https://doi.org/10.1101/2021.01.25.428137

This article is a preprint and has not been certified by peer review [what does this mean?].

0 0 0 0 100 1032

Abstract Full Text Info/History Metrics Preview PDF

Abstract

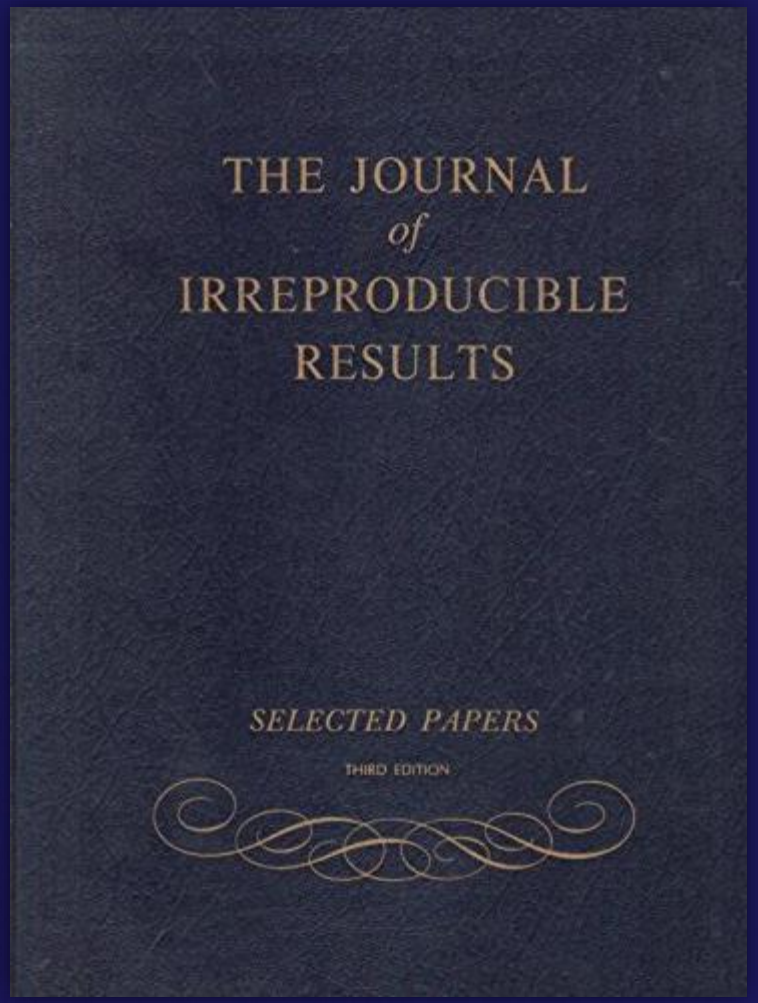
The COVID-19 pandemic has ravaged the globe, and its causative agent, SARS-CoV-2, continues to rage. Prospects of ending this pandemic rest on the development of effective interventions. Single and combination monoclonal antibody (mAb) therapeutics have received emergency use authorization[1–3], with more in the pipeline[4–7]. Furthermore, multiple vaccine constructs have shown promise[8], including

Pseudo-Viruses

- "… we created VSV-based SARS-78 CoV-2 pseudoviruses that contain each of the individual mutations as well as one with all 8 mutations of the B.1.1.7 variant (UKΔ8) and another with all 9 mutations of the B.1.351 variant (SAΔ9). A total of 18 mutant pseudoviruses were made as previously described (20,21), and each was found to have a robust titer (Extended Data Fig. 1) adequate for neutralization studies."

Delta Variant

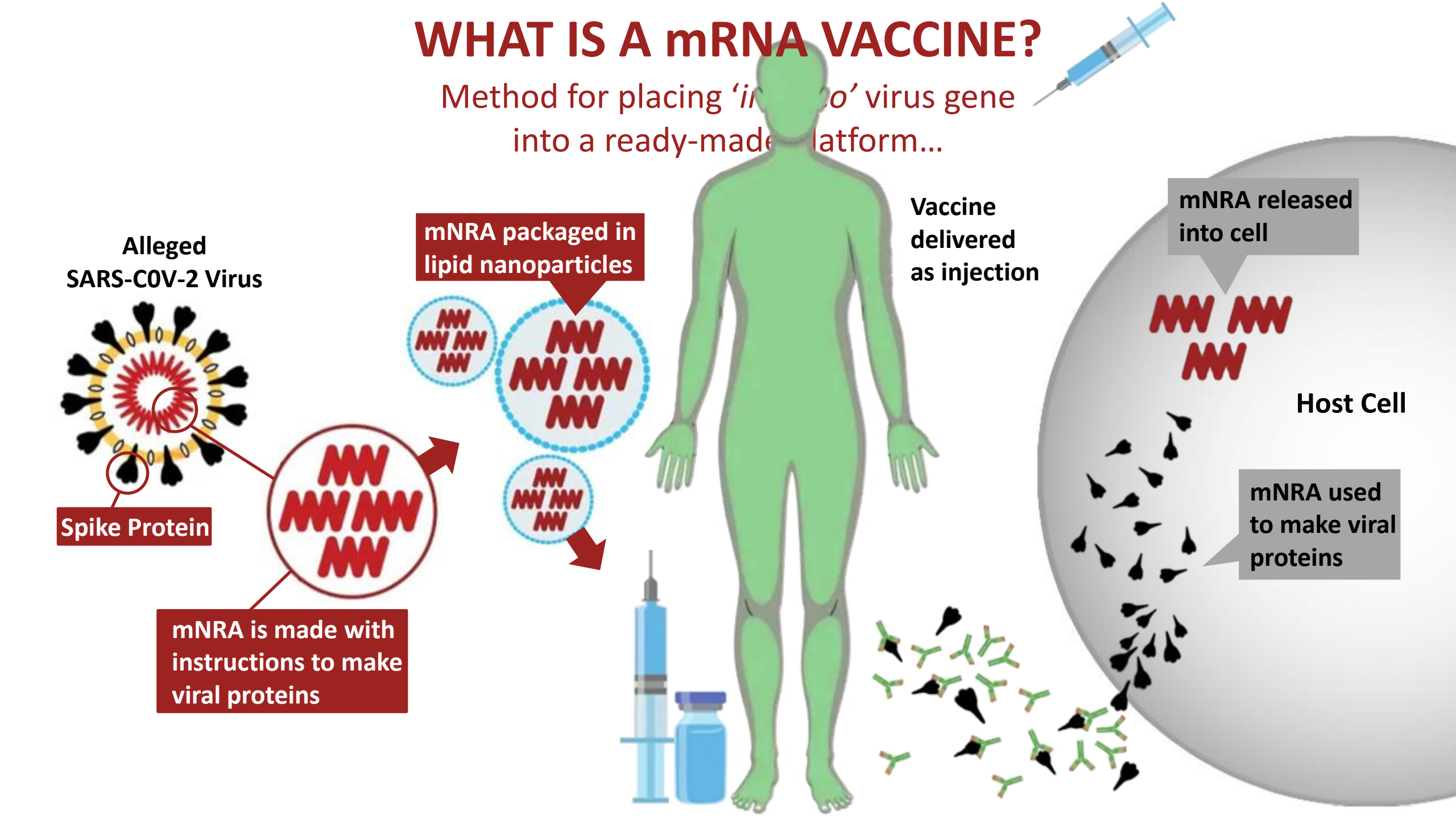
- No known attempt to isolate!!! Does not exist in reality!
- Discovered only by in silico genome sequencing
- Clinical properties only studied by
  - Monoclonal antibodies
  - Pseudoviruses
  - Computer modeling
- There is no clinical test authorized, approved, or available for purchase for any variant!!!

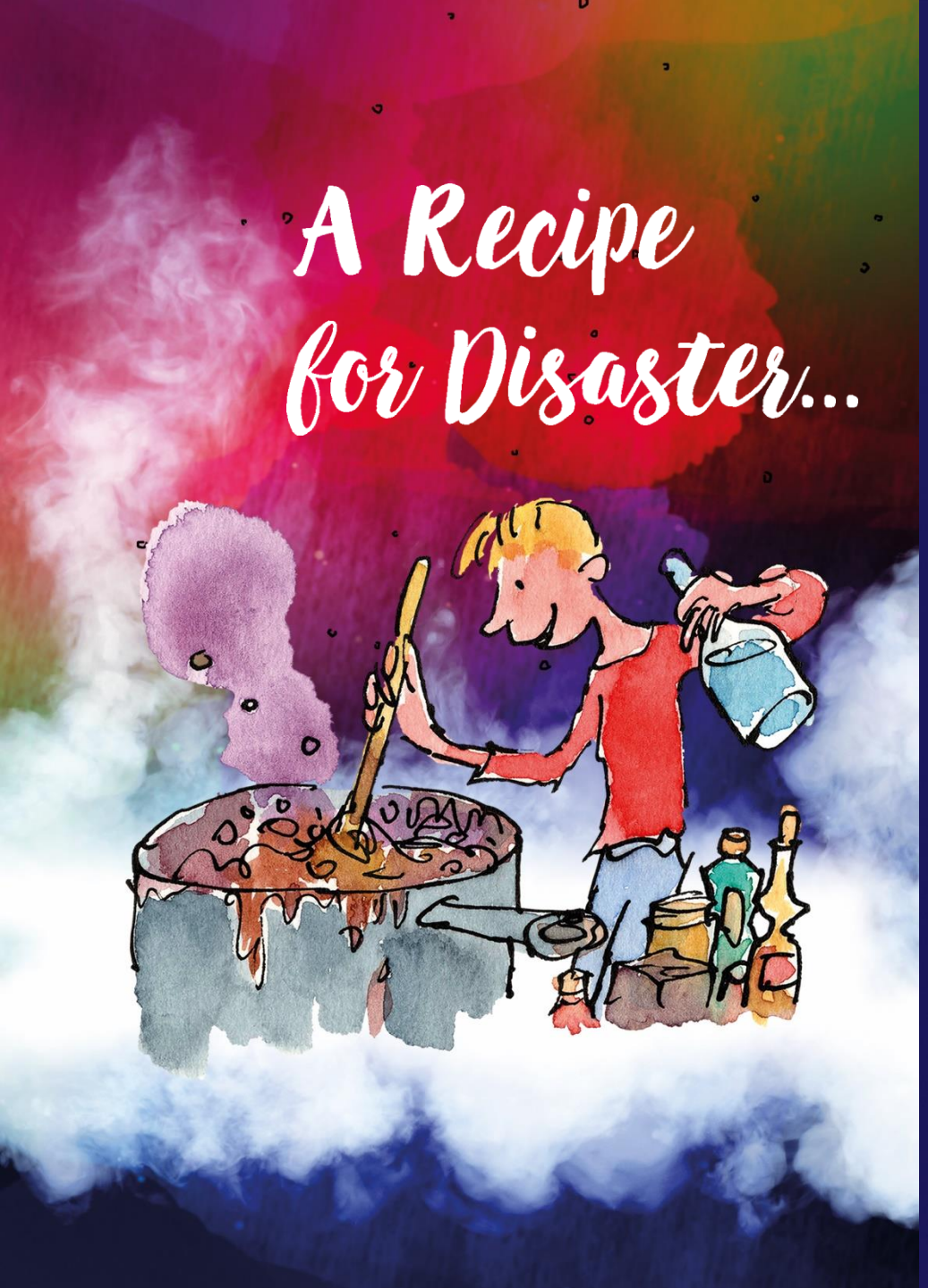


Variants are simply the inability to reproduce or validate the original results.

WHAT IS A mRNA VACCINE?

Method for placing '*in* *to*' virus gene into a ready-made platform…





Pandemic Virus Recipes

- First Generation
  - Proof of new virus: “virus isolation”
  - Vaccine: toxic tissue culture filtrate
  - FDA approval: ≥ 10 years
- Next (covid) Generation
  - Proof of new virus: ‘*in silico*’ genome
  - Vaccine: “viral gene” in delivery vehicle (pre-made)
  - FDA EUA: < 1 year

Summary

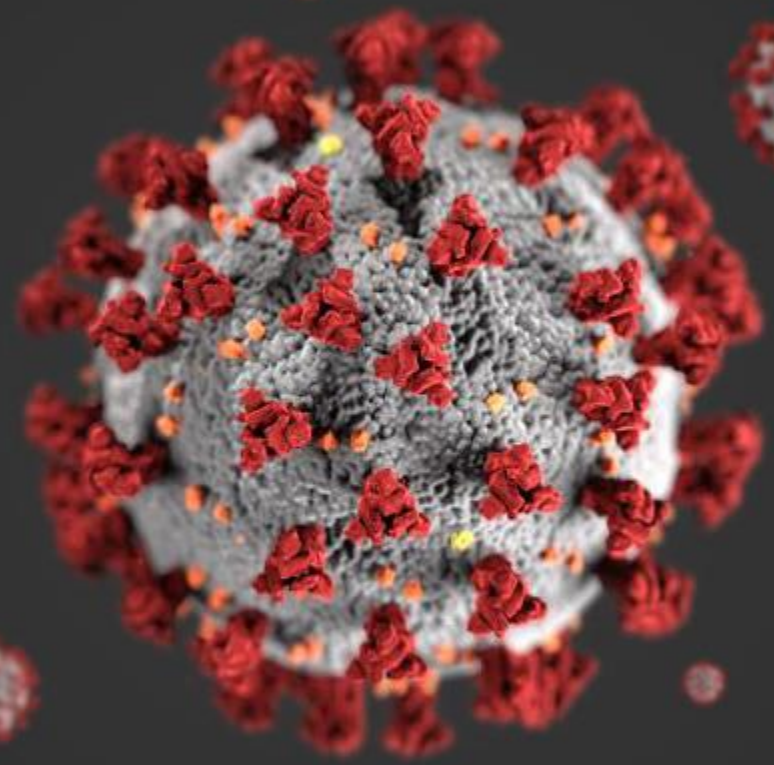
- A genome is the full code of an organism
- An *in silico* genome is a theoretical construct
- The new paradigm of virus pandemic creation:
  - Mimic the symptoms of another disease
  - Perform tissue culture “isolation” (soon obsolete)
  - Create an ‘*in silico*’ genome
  - PCR test based on above
  - Manufacture gene therapy “vaccine”

PATHOGENIC PRIMING, ANTIBODY DEPENDENT ENHANCEMENT

What does the science show?

FACT No. 1

SARS-CoV-2 Does NOT EXIST!!



Computer generated, 3D Biomedical Art
by artists Alissa Eckert, and Dan Higgins
To Illustrate ultrastructural morphology
alleged to be exhibited by coronaviruses.

- "One potential hurdle for antibody-based vaccines and therapeutics is the risk of exacerbating COVID-19 severity via antibody-dependent enhancement (ADE)."
- "No definitive role for ADE in human coronavirus diseases has been established."

nature microbiology

PERSPECTIVE

https://doi.org/10.1038/s41564-020-00789-5

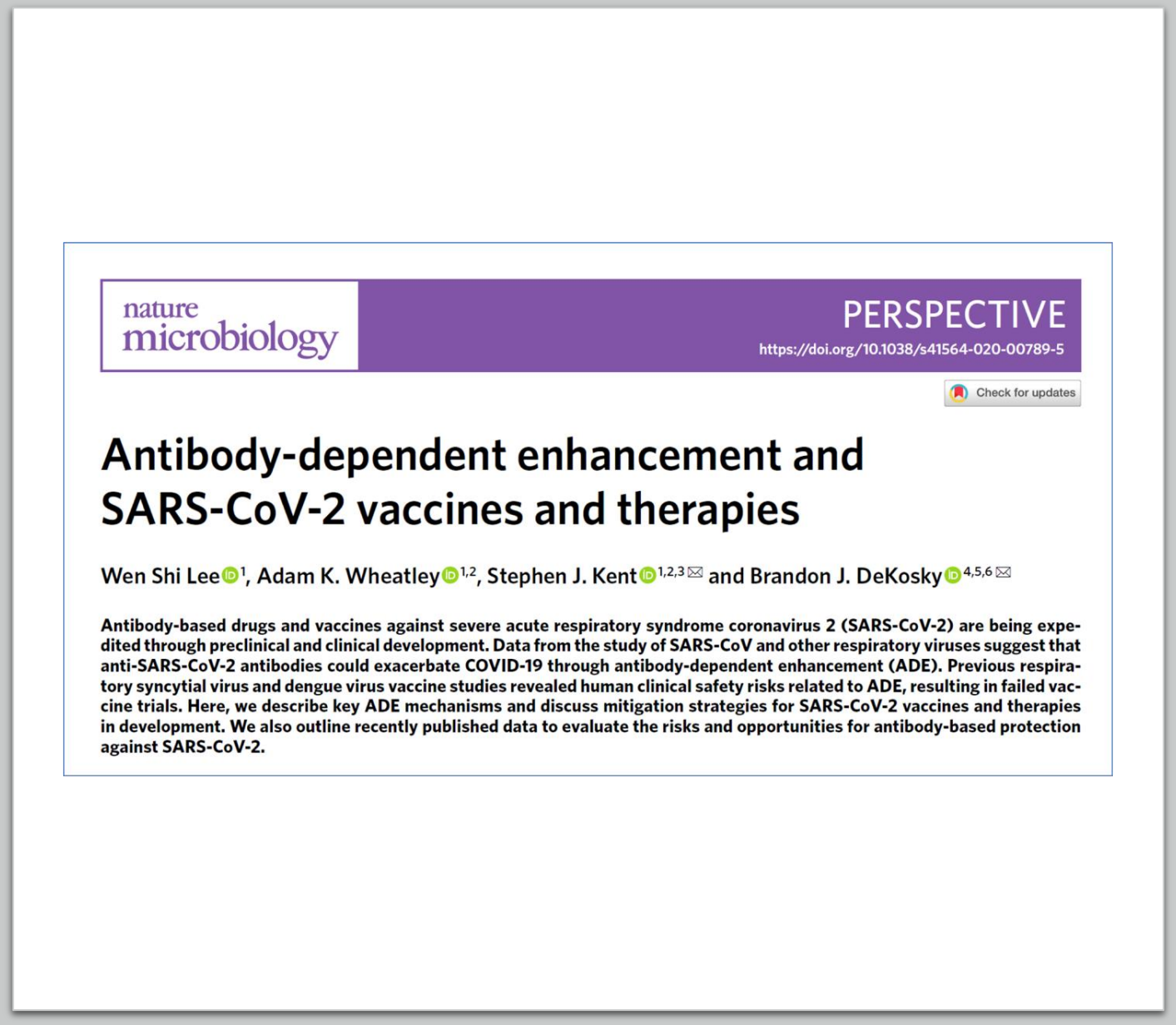
Check for updates

Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies

Wen Shi Lee[1], Adam K. Wheatley[1,2], Stephen J. Kent[1,2,3] ✉ and Brandon J. DeKosky[4,5,6] ✉

Antibody-based drugs and vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are being expedited through preclinical and clinical development. Data from the study of SARS-CoV and other respiratory viruses suggest that anti-SARS-CoV-2 antibodies could exacerbate COVID-19 through antibody-dependent enhancement (ADE). Previous respiratory syncytial virus and dengue virus vaccine studies revealed human clinical safety risks related to ADE, resulting in failed vaccine trials. Here, we describe key ADE mechanisms and discuss mitigation strategies for SARS-CoV-2 vaccines and therapies in development. We also outline recently published data to evaluate the risks and opportunities for antibody-based protection against SARS-CoV-2.

"ADE has been observed in SARS, MERS and other human respiratory virus infections including RSV and measles, which suggests a real risk of ADE for SARS-CoV-2 vaccines and antibody-based interventions. However, clinical data has not yet fully established a role for ADE in human COVID-19 pathology."



nature microbiology

PERSPECTIVE

https://doi.org/10.1038/s41564-020-00789-5

Check for updates

Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies

Wen Shi Lee[1], Adam K. Wheatley[1,2], Stephen J. Kent[1,2,3] ✉ and Brandon J. DeKosky[4,5,6] ✉

Antibody-based drugs and vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are being expedited through preclinical and clinical development. Data from the study of SARS-CoV and other respiratory viruses suggest that anti-SARS-CoV-2 antibodies could exacerbate COVID-19 through antibody-dependent enhancement (ADE). Previous respiratory syncytial virus and dengue virus vaccine studies revealed human clinical safety risks related to ADE, resulting in failed vaccine trials. Here, we describe key ADE mechanisms and discuss mitigation strategies for SARS-CoV-2 vaccines and therapies in development. We also outline recently published data to evaluate the risks and opportunities for antibody-based protection against SARS-CoV-2.

"Autopsies of Chinese citizens who have died from COVID-19 following SARS-CoV-19 infection show evidence of interstitial changes, **suggesting** the development of pulmonary fibrosis [1]. This suggests, at least partly, an autoimmunology basis of the pathogenesis of COVID-19."

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Pathogenic priming likely contributes to serious and critical illness and mortality in COVID-19 via autoimmunity

James Lyons-Weiler

The Institute for Pure and Applied Knowledge, USA

ARTICLE INFO

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ABSTRACT

Homology between human and viral proteins is an established factor in viral- or vaccine-induced autoimmunity. Failure of SARS and MERS vaccines in animal trials involved pathogenesis consistent with an immunological priming that could involve autoimmunity in lung tissues due to previous exposure to the SARS and MERS spike protein. Exposure pathogenesis to SARS-CoV-2 in COVID-19 likely will lead to similar outcomes. Immunogenic peptides in viruses or bacteria that match human proteins are good candidates for pathogenic priming peptides (similar to the more diffuse idea of "immune enhancement"). Here I provide an assessment of potential for human pathogenesis via autoimmunity via exposure, via infection or injection. SAR-CoV-2 spike proteins, and all other SARS-CoV-2 proteins, immunogenic epitopes in each SARS-CoV-2 protein were compared to human proteins in search of high local homologous matching. Only one immunogenic epitope in a SARS-CoV-2 had no homology to human proteins. If all of the parts of the epitopes that are homologous to human proteins are excluded from consideration due to risk of pathogenic priming, the remaining immunogenic parts of the epitopes may be still immunogenic and remain as potentially viable candidates for vaccine development. Mapping of the genes encoding human protein matches to pathways point to targets that could explain the observed presentation of symptoms in COVID-19 disease. It also strongly points to a large number of opportunities for expected distur-

[1] H. Shi, et al., Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study, Lancet (2020)

Causes of Pulmonary Fibrosis

Occupational and environmental factors

- Silica dust, asbestos, metal dusts, coal dust, grain dust, bird/animal droppings

Radiation treatments

Medications

- Chemotx, anti-arrythmics, antibiotics, immunosuppressive antibodies

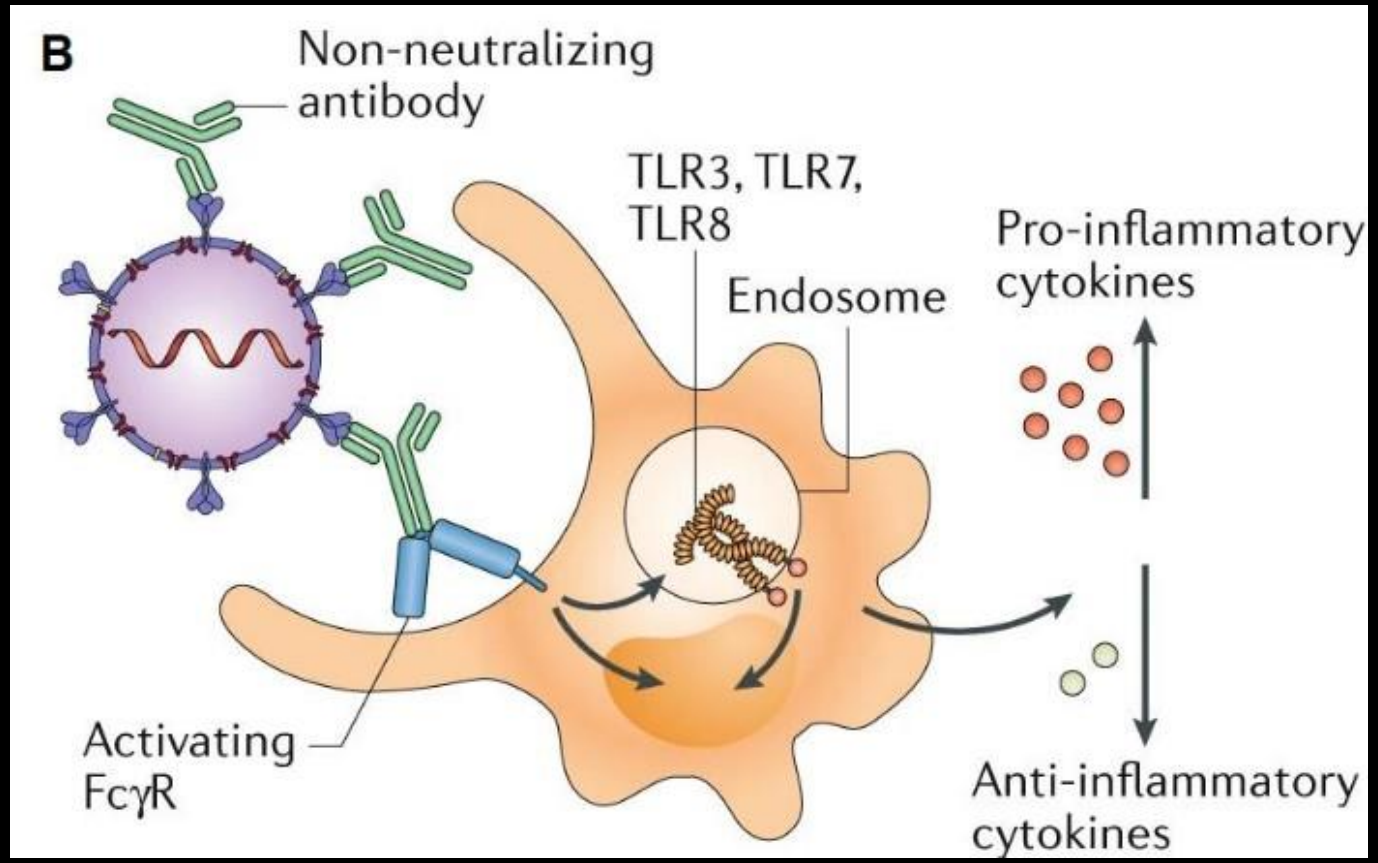
Medical conditions

- Connective tissue disorders, rheumatoid arthritis, sarcoidosis, scleroderma, lupus, **pneumonia**

https://www.mayoclinic.org/diseases-conditions/pulmonary-fibrosis/symptoms-causes/syc-20353690

THE STUDIES…

Is there evidence for ADE?



RESPIRATORY SYNCYTIAL VIRUS DISEASE IN INFANTS DESPITE PRIOR ADMINISTRATION OF ANTIGENIC INACTIVATED VACCINE[1, 2]

HYUN WHA KIM, JOSE G. CANCHOLA[3], CARL D. BRANDT, GLORIA PYLES, ROBERT M. CHANOCK, KEITH JENSEN, AND ROBERT H. PARROTT[4]

(Received for publication August 8, 1968)

Kim, H. W., J. G. Canchola, C. D. Brandt, G. Pyles, R. M. Chanock, K. Jensen and R. H. Parrott (Children's Hosp. of D.C., Wash., D.C. 20009). Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Amer. J. Epid.*, 1969, 89: 422–434.—In response to three injections of alum precipitated, 100X concentrated, formalin inactivated RS vaccine (lot 100), 43% of infant vaccinees displayed a 4-fold or greater rise in serum neutralizing antibody and 91% displayed a 4-fold or greater rise in serum CF antibody. When RS virus became prevalent in the community, the rate of RS virus infection in infants who received this vaccine was not remarkably different from that in control infants who received parainfluenza vaccines. However, 80% of RS vaccinees required hospitalization at the time of RS infection

Study Design: RSV

54 infants received RSV vaccine, 3 injections

- Toxic cell culture derived, monkey and human embryonic kidney cells
- Formaldehyde added
- Concentrated 4X and precipitated with aluminum (alum)
- Antibiotics added: polymixin B, streptomycin, neomycin
- Benzethonium chloride preservative (seizures, tumors, CNS)

Control: parainfluenza vaccine

- No antibiotics or preservatives added

Followed to observe incidence RSV infection

TABLE 5

RS virus infection and serious illness in comparable groups of infants receiving one or more injections of inactivated RS and parainfluenza vaccines

Vaccine	Category of infants	No. and age of infants during designated time period of RS virus prevalence				Total No. infants
		1965–1966		1966–1967		
		No. infants	Age§ (mo.)	No. infants	Age§ (mo.)	
RS lot 100	At risk*	20	5.1	25‡	12.7	31
	RS infection†	5		15		20 (65%)‖
	Hospitalized	4		12		16 (80%)¶
Para 1 lot 23	At risk*	20	5.0	17‡	15.8	20
	RS infection†	2		10		12 (60%)‖
	Hospitalized			1		1 (8%)¶
Trivalent parainfluenza lot 6279	At risk*			20	8.4	20
	RS infection†			9		9 (45%)‖
	Hospitalized			0		0
Total parainfluenza	At risk*	20	5.0	37	11.8	40
	RS infection†	2		19		21 (53%)‖
	Hospitalized			1		1 (5%)¶

* No prior natural infection.
† As indicated by 4-fold or greater CF antibody rise only; reinfection counted only once.
‡ One infant was not available to follow up.
§ Mean age at peak of RS prevalence.
‖ Per cent of infants at risk who sustained RS infection.
¶ Per cent of infected individuals who were hospitalized.

Author Conclusions: Valid?

Vaccine did NOT prevent RSV infection

No increased susceptibility to RSV

Increased clinical severity of RSV

- "We lack a definite explanation for this phenomenon…"
- Antibodies may play a role

Validity

- Small study size
- Not randomized
- Improper control intervention
- Did not consider direct toxicity of vaccine, antibiotics, preservatives

- 11 children with atypical acute illness with rash in small town
- All the children were exposed to "measles"
- 4 years earlier they were subjects in measles vaccine study
- "Little is currently known about the etiology of such reactions, or the basic immunologic mechanisms involved."
- Validity
  - Small sample
  - Case control design, not RCT
  - 4-year delay suggests not related to vaccine
  - Small geographic region suggests common exposure to toxin

January, 1968
The Journal of PEDIATRICS

Atypical exanthem following exposure to natural measles: Eleven cases in children previously inoculated with killed vaccine

A severe illness characterized by high fever, tachypnea, myalgia, prostration, and an atypical exanthem occurred after exposure to measles in children previously immunized with killed measles vaccine. Laboratory and epidemiological data were suggestive of recent measles infection. Other previously immunized children had local reactions at the site of injection of live measles vaccine. The host response to subsequent challenge with live measles virus is apparently sometimes altered by prior vaccination with inactivated measles vaccine, but the exact mechanisms remain obscure.

Philip R. Nader, M.D.,* Marshall S. Horwitz, M.D., and John Rousseau, M.D.

ATLANTA, GA., AND RIVERTON, WYO.

- Naturally or artificially "infected" immunized cats with 2 strains of FIPV
- No clinical information
- Antibody studies in cell culture only
- Found "ADE" in one strain only – what does it mean?
- What happened to the cats?
- How were they infected?
- What vaccines did they receive?

Antibody-Dependent Enhancement of Feline Infectious Peritonitis Virus Infection in Feline Alveolar Macrophages and Human Monocyte Cell Line U937 by Serum of Cats Experimentally or Naturally Infected with Feline Coronavirus

Tsutomu HOHDATSU, Mika YAMADA, Ritsuko TOMINAGA, Kaori MAKINO, Kouji KIDA and Hiroyuki KOYAMA

Department of Veterinary Infectious Diseases, School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada, Aomori 034, Japan

(Received 3 July 1997/Accepted 28 August 1997)

ABSTRACT. Infection of the type II feline infectious peritonitis virus (FIPV) strain 79–1146 to primary feline alveolar macrophages and human monocyte cell line U937 was enhanced by the sera of cats experimentally infected with the 79–1146 strain, but not those of cats infected with KU-2 or UCD-1 strain of type I FIPV. The experiments using sera of cats with feline infectious peritonitis (FIP) and of cats naturally infected with feline coronavirus (FCoV) revealed that infection of the FIPV 79–1146 strain to the U937 cells was enhanced only by the sera of cats infected with type II FIPV or feline enteric coronavirus. The samples positive for antibody-dependent enhancement (ADE) activity had high neutralizing antibody titers against the FIPV 79–1146 strain and the samples negative for ADE activity had low neutralizing antibody titers. These findings support the previous results where a monoclonal antibody with neutralizing activity had high ADE activity, suggesting that there was a close relationship between the neutralization and enhancement sites. And then it is also suggested that ADE of infection is likely to be induced by re-infection with the same serotype of virus in type II FIPV infection. Furthermore, U937 cells are considered useful and can be substituted for the feline macrophages for determining ADE of FIPV-infection.
— KEY WORDS: antibody-dependent enhancement of infection, feline infectious peritonitis virus, feline macrophage, U937 cell.

J. Vet. Med. Sci. 60(1): 49–55, 1998

JOURNAL OF VIROLOGY, Nov. 2004, p. 12672–12676 Vol. 78, No. 22
0022-538X/04/$08.00+0 DOI: 10.1128/JVI.78.22.12672–12676.2004

Immunization with Modified Vaccinia Virus Ankara-Based Recombinant Vaccine against Severe Acute Respiratory Syndrome Is Associated with Enhanced Hepatitis in Ferrets

Hana Weingartl,[1,2]† Markus Czub,[2,3]† Stefanie Czub,[1,2] James Neufeld,[1] Peter Marszal,[1] Jason Gren,[1] Greg Smith,[1] Shane Jones,[3] Roxanne Proulx,[3] Yvonne Deschambault,[3] Elsie Grudeski,[3] Anton Andonov,[2,3] Runtao He,[2,3] Yan Li,[2,3] John Copps,[1] Allen Grolla,[3] Daryl Dick,[3] Jody Berry,[1,2] Shelley Ganske,[1] Lisa Manning,[1] and Jingxin Cao[2,3]*

National Centre for Foreign Animal Diseases[1] and National Microbiology Laboratory,[3] Canadian Science Centre for Human and Animal Health, Winnipeg, Manitoba, Canada R3E 3R2, and Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2[2]

Received 29 April 2004/Accepted 29 July 2004

“Thus, our data suggest that vaccination with rMVA expressing SARS-CoV S protein is associated with enhanced hepatitis.”

Study Design: SARS

- Recombinant modified vaccinia virus Ankara (rMVA) expressing SARS-CoV S protein (rMVA-S) – similar to J&J
- 10-week-old castrated ferrets in solitary confinement, GMO food
- Control group 1: vaccine MVA (ferrets 1 to 3)
- Ferrets 4 to 6 ???
- Experimental group: rMVA-S (ferrets 7 to 9)
- Control group 2: PBS (ferrets 10 to 12)
- Day 0 and 14 by intraperitoneal and subcutaneous routes
- 14d later, ferrets challenged with SARS-CoV Tor2 isolate (intranasal)

What happened to the poor ferrets?

"…no clinical signs (e.g., elevated temperature and altered behavior including feeding) were observed up to 29 days post challenge…"

Transient elevation in AST, other LFTs normal

Animals sacrificed day 27 to 29

"…it is likely that the liver inflammation…in fact, may represent the recovering stage."

"All groups of ferrets showed clinical signs after challenge, indicating that ferrets became infected with SARS-CoV and that none of the vaccines tested blocked infection. All ferrets had increased temperatures after challenge, but significant differences between groups were not seen…"

"There was no apparent evidence of enhanced liver pathology…" (no intraperitoneal injections)

"Gross pathology indicated frequent significant hemorrhage in lung and mediastinum (the area between the lungs containing the heart, trachea, esophagus, thymus and lymph nodes), specifically the thymus (Fig. 5), but other organs appeared largely normal."

"Although all ferrets were sacrificed by day 6 post-challenge, our results did not suggest vaccine-induced immune enhancement of disease in any tissues."

Journal of General Virology (2008), 89, 2136–2146 DOI 10.1099/vir.0.2008/001891-0

Severe acute respiratory syndrome vaccine efficacy in ferrets: whole killed virus and adenovirus-vectored vaccines

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Although the 2003 severe acute respiratory syndrome (SARS) outbreak was controlled, repeated transmission of SARS coronavirus (CoV) over several years makes the development of a SARS vaccine desirable. We performed a comparative evaluation of two SARS vaccines for their ability to protect against live SARS-CoV intranasal challenge in ferrets. Both the whole killed SARS-CoV vaccine (with and without alum) and adenovirus-based vectors encoding the nucleocapsid (N) and spike (S) protein induced neutralizing antibody responses and reduced viral replication and shedding in the upper respiratory tract and progression of virus to the lower respiratory tract. The vaccines also diminished haemorrhage in the thymus and reduced the severity and extent of pneumonia and damage to lung epithelium. However, despite high neutralizing antibody titres, protection was incomplete for all vaccine preparations and administration routes. Our data suggest that a combination of vaccine strategies may be required for effective protection from this pathogen. The ferret may be a good model for SARS-CoV infection because it is the only model that replicates the fever seen in human patients, as well as replicating other SARS disease features including infection by the respiratory route, clinical signs, viral replication in upper and lower respiratory tract and lung damage.

Received 6 March 2008
Accepted 1 May 2008

NO FERRETS DIED IN THE MAKING OF THIS PRESENTATION (they were killed by the scientists)

- "Six- to eight-week-old, female Balb/c and C57BL/6 mice…were housed in cages covered with barrier filters…"
- Given a variety of SARS vaccines and challenged with "SARS"
- Sacrificed 2 days later, no clinical outcomes reported (none sick)
- "…all animals exhibited pathologic changes after challenge…"

OPEN ACCESS Freely available online

PLoS one

Immunization with SARS Coronavirus Vaccines Leads to Pulmonary Immunopathology on Challenge with the SARS Virus

Chien-Te Tseng[1,2], Elena Sbrana[1], Naoko Iwata-Yoshikawa[1,2], Patrick C. Newman[1], Tania Garron[1], Robert L. Atmar[3,4], Clarence J. Peters[1,2], Robert B. Couch[3,4]*

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Abstract

Background: Severe acute respiratory syndrome (SARS) emerged in China in 2002 and spread to other countries before brought under control. Because of a concern for reemergence or a deliberate release of the SARS coronavirus, vaccine development was initiated. Evaluations of an inactivated whole virus vaccine in ferrets and nonhuman primates and a virus-like-particle vaccine in mice induced protection against infection but challenged animals exhibited an immunopathologic-type lung disease.

Design: Four candidate vaccines for humans with or without alum adjuvant were evaluated in a mouse model of SARS, a VLP vaccine, the vaccine given to ferrets and NHP, another whole virus vaccine and an rDNA-produced S protein. Balb/c or C57BL/6 mice were vaccinated IM on day 0 and 28 and sacrificed for serum antibody measurements or challenged with live virus on day 56. On day 58, challenged mice were sacrificed and lungs obtained for virus and histopathology.

Results: All vaccines induced serum neutralizing antibody with increasing dosages and/or alum significantly increasing responses. Significant reductions of SARS-CoV two days after challenge was seen for all vaccines and prior live SARS-CoV. All mice exhibited histopathologic changes in lungs two days after challenge including all animals vaccinated (Balb/C and C57BL/6) or given live virus, influenza vaccine, or PBS suggesting infection occurred in all. Histopathology seen in animals given one of the SARS-CoV vaccines was uniformly a Th2-type immunopathology with prominent eosinophil infiltration, confirmed with special eosinophil stains. The pathologic changes seen in all control groups lacked the eosinophil prominence.

Conclusions: These SARS-CoV vaccines all induced antibody and protection against infection with SARS-CoV. However, challenge of mice given any of the vaccines led to occurrence of Th2-type immunopathology suggesting hypersensitivity to SARS-CoV components was induced. Caution in proceeding to application of a SARS-CoV vaccine in humans is indicated.

Citation: Tseng C-T, Sbrana E, Iwata-Yoshikawa N, Newman PC, Garron T, et al. (2012) Immunization with SARS Coronavirus Vaccines Leads to Pulmonary Immunopathology on Challenge with the SARS Virus. PLoS ONE 7(4): e35421. doi:10.1371/journal.pone.0035421

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Competing Interests: The authors have declared that no competing interests exist.

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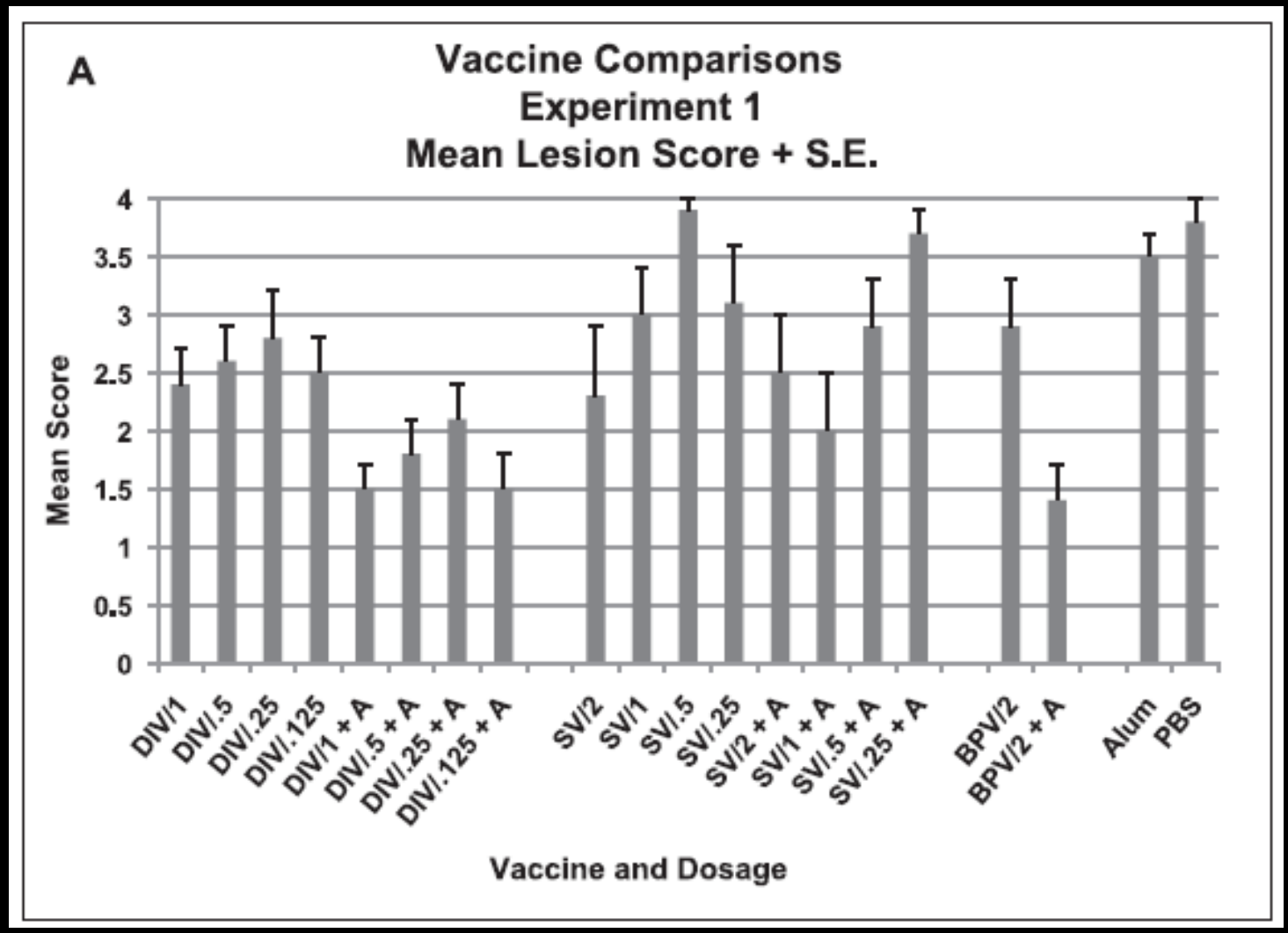
- DIV – Double Inactivated Vaccine
- VLP – Virus-Like Particle vaccine
- SV – recombinant Spike Vaccine
- BPV – Beta-Propionolactone Vaccine

- Lung Pathology
  - "Analyses: A. Mean lesion scores were different p,.001. DIV without alum greater than with alum p = .001, VLP without alum greater than with alum p = .008. Post-hoc comparisons: DIV lower than SV p = .001 and controls p,.001 but not VLP p..05. SV lower than controls p .048."



- Transgenic mice, groups of 6
- WIV – Whole Inactivated Virus vaccine
- Two IM injections, 3 weeks apart
- Groups
  - WIV alone
  - WIV plus aluminum hydrogel
  - WIV plus MF59 adjuvant - *muramyl tripeptide linked to dipalmitoyl phosphatidyl ethanolamine*
  - Control 1 – alum only
  - Control 2 – MF59 only
- Three weeks later challenged with MERS-CoV (intranasally)
- Sacrificed day 3 or 6
- No clinical outcomes!!

HUMAN VACCINES & IMMUNOTHERAPEUTICS
2016, VOL. 12, NO. 9, 2351–2356
http://dx.doi.org/10.1080/21645515.2016.1177688

Taylor & Francis
Taylor & Francis Group

SHORT REPORT

Immunization with inactivated Middle East Respiratory Syndrome coronavirus vaccine leads to lung immunopathology on challenge with live virus

Anurodh Shankar Agrawal[a], Xinrong Tao[a], Abdullah Algaissi[a,e], Tania Garron[a], Krishna Narayanan[a], Bi-Hung Peng[b], Robert B. Couch[c], and Chien-Te K. Tseng[a,d]

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ABSTRACT

To determine if a hypersensitive-type lung pathology might occur when mice were given an inactivated MERS-CoV vaccine and challenged with infectious virus as was seen with SARS-CoV vaccines, we prepared and vaccinated mice with an inactivated MERS-CoV vaccine. Neutralizing antibody was induced by vaccine with and without adjuvant and lung virus was reduced in vaccinated mice after challenge. Lung mononuclear infiltrates occurred in all groups after virus challenge but with increased infiltrates that contained eosinophils and increases in the eosinophil promoting IL-5 and IL-13 cytokines only in the vaccine groups. Inactivated MERS-CoV vaccine appears to carry a hypersensitive-type lung pathology risk from MERS-CoV infection that is similar to that found with inactivated SARS-CoV vaccines from SARS-CoV infection.

ARTICLE HISTORY
Received 8 February 2016
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Accepted 7 April 2016

KEYWORDS
coronavirus; Eosinophils; immunopathology; Middle East Respiratory Syndrome; vaccination

"No gross pathology was noted on either day 3 or 6 (data not shown); however, histopathology was noted in all groups on both days."

TRANSGENIC MICE

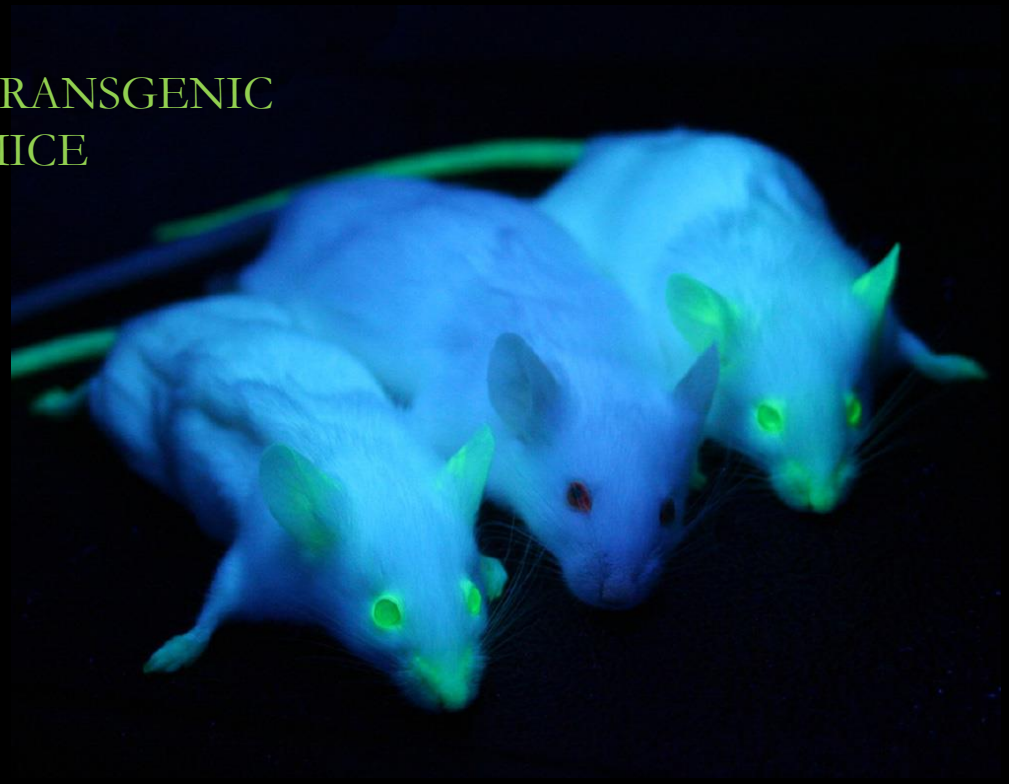

Table 1. Severity of lung histopathology of vaccinated mice after challenge with MERS-CoV.

	Severity score of lung pathology*	
Vaccination Groups	Day 3**	Day 6
Alum only	1	1
MF59 only	1	1
WIV/Alum	2	2
WIV/MF59	2	2
WIV only	2	2

* Pathology severity scores (0-3): 0- no pathology, 1- mild, 2- moderate, and 3- severe.
**Day post challenge.

A Rumor of a Shadow of a Fingerprint

- No animals died from vaccination followed by challenge with toxic cell culture
- When clinical outcomes were observed, there was no difference between vaccinated and control groups that supported ADE/PP
- Results suggest vaccines and cell culture supernatants are sub-clinically to clinically toxic
- Human cohort studies suggest vaccines are toxic
- Antibody and cell culture experiments based upon false premise, should disregard

...Graphic Artist at Work!