Origin of the Covid-19 virus: update April 1, 2022

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The origin of the Covid-19 virus

Summary

The unnatural origin of SARS-CoV-2 has been raised and discussed for 2 years. It is important to talk about it to understand the inflection of biopolitics since the 2000s.
In addition, structural features of the virus, new compared to other known coronaviruses, may explain certain aspects of the clinical and therapeutic aspects of Covid-19.

SARS-CoV-2 is the only human pathogenic coronavirus to possess a polybasic furin cleavage site and a human ACE2 binding site at the same time
which explain its ability to infect humans and its pathogenicity.
The main argument against the natural origin of the virus is that no animal that acted as an intermediate host could be identified and no related virus from which it could have evolved naturally was found. In favor of the "artificial" or "synthetic" origin, mention should be made (among other arguments) of the experiments carried out in the past on the insertion of a furin site and of a human ACE2 binding site, as well as projects revealed by recently declassified documents. SARS-CoV-2 also has the ability to bind to other receptors that some gain-of-function experiments might have sought to optimize.

These gain-of-function (GoF) experiments are described very precisely in EcoHealthAlliance's response to a DARPA (US Army Research Agency) call for tenders. Coronavirus GoFs began to be funded by the NIH in the early 2000s and involved the Wuhan Laboratory (WIV) thereafter. The European Commission also finances the WIV with the Horizon 2020 project (EVAg and EVA Global).

An investigation is underway in the United States Senate and senators have said that the leak of a laboratory was the most likely option and spoke of gains in function led by the NIH in Wuhan despite the moratorium that would have been circumvented.

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Update April 1 , 2022

Definitions: natural or non-natural origin

For two years the question of the natural origin or not of the Covid-19 virus has been fiercely discussed. What do the terms “natural” and “artificial” mean with regard to the origin of a virus?

Natural origin: it is a zoonosis virus capable of infecting humans and causing a pandemic, therefore possessing the ability to be transmitted immediately and very effectively from human to human
In the case of MERS1 and Ebola2 , there are sporadic epidemics by "spillover" overflow, but no pandemic.
The SARS-CoV epidemic may have started with several introductions from the wild animal, the civet3 .

Artificial or synthetic origin: it is a virus originating from the bat which has been cultivated in laboratory (on cell lines and in animals) and which escapes from the laboratory.
This virus may have undergone a voluntary modification (human intervention to modify its sequence, or even total synthesis from a modified sequence compared to those known) or involuntary (by passages on cell cultures). In all cases there is an obligatory passage on cells in culture.

The latest developments in the controversy

Recently important documents were declassified in the United States at the end of 2021 and the US Congress seized these documents and asked specific questions of the government.

On March 5, 2022 The Economist headlined 2 articles that would provide evidence of the natural origin of the virus and emergence at the Wuhan wildlife market4 . This article is the last of a long series trying to prove this natural origin but without ever providing proof. More particularly that of Worobey *et al.* [5], dates its survey back to mid-December 2019, while many clues point to the emergence of the virus several months earlier (two European serological surveys find a seroprevalence significant from November 20196 . We will see that strong pressure has been exerted towards publications suggesting a non-natural origin of the virus or the possibility that SARS-CoV-2 has structural and functional homologies with HIV: these publications have been withdrawn by their authors in order to escape future trouble!

More generally, it is important to ask the question of the origin of the Covid-19 virus from a biopolitical point of view (a concept developed by Michel Foucault in the late 1970s): it is politics specific to the population aimed at optimizing its reproduction and productivity. From an economic point of view, the state of health of the employed and employable population must be normalized even if it is to the detriment of individuals (La Naissance de la biopolitique. Cours au Collège de France (1978-1979) and Sécurité , Territory, Population (1978) , Le Seuil- 2004). We will see that to the biological normalization of human populations is added that of wild animal populations.

In order to protect populations, in the same way that States seek to anticipate economic crises with bank stress tests, they launch research programs to anticipate and prevent
pandemics with emerging viruses: in particular PREDICT from USAID (US Agency for International Development) and PREEMPT from DARPA (Defense Advanced Research Projects Agency)7 and EVAg and EVA from the European Commission which must, among other goals, make it possible to anticipating the response to emerging viral diseases8
Vaccination has long been a means of this health biopolitics.
In 1760, the mathematician Bernoulli already declared: “If we adopt inoculation, the result will be a gain of several thousand people for civil society; even if it is murderous, as it kills infants in the cradle, it is preferable to smallpox which kills adults who have become useful to society..., Bernouilli concluded that, if we neglect the point of view of individual, "it will always be geometrically true that the interest of Princes is to promote inoculation. »9

Since the 2000s, biopolitics has focused on generalized vaccination. The WHO declared the eradication of smallpox in 1980 after observing the last case in 197710 and in 1978 the CDC set the goal of eliminating measles by 1982. 11The UN also hopes for vaccination coverage 100% of the world's population by 203012.

In 2018 the Council of the European Union proposes to strengthen cooperation against vaccine-preventable diseases13. This involves increasing vaccination coverage (VC), standardizing vaccination schedules and creating a the EU , European vaccination record. Before the Covid-19 pandemic, measles was the spearhead of this policy with a target of 95% CV by 2020, thus meeting the WHO objectives. Of the

Experts are concerned in 2021 about the exaggerated importance of the industrial complex of the vaccine industry, which risks relegating science to the background behind the economy. [14]

The first doubts about the origin of the virus

The first doubts appeared in early 2020 with the publication of the SARS-CoV-2 genome: virologists noticed molecular characteristics that suddenly appeared on this virus compared to previously known coronaviruses.

Specialists in the HIV virus have noticed sequence homologies with this virus and the presence of the furin site has jumped out at coronavirologists.
The history of gain-of-function experiments on these viruses as well as the experiments described in the EcoHealth Alliance (EHA) DEFUSE project responding to a DARPA call for tenders under PREDICT (see below) reinforce the hypothesis of a synthetic origin without however proving it formally. The DEFUSE project consisted of anticipating a coronavirus pandemic by building a dangerous virus that could emerge and at the same time designing the vaccine and the therapeutics to fight it. The chimeric virus described in DEFUSE has several key molecular characteristics. These same characteristics are found in the SARS-CoV-2 which has really emerged and they pose a problem clinically and therapeutically, even when the virus has attenuated and become endemic as at the start of 2022. In particular, the furin site allows the viruses to infect humans and enter many organs; moreover it is a superantigen which causes specific immunopathological effects compared to other coronaviruses 15

infecting humans.

Knowledge of the molecular characteristics of SARS-CoV-2 is therefore important from a clinical and therapeutic.

A bit of virology

The furin site and the human ACE2 receptor

SARS-CoV-2 is an enveloped virus: the genetic material (RNA) bound to the nucleocapsid protein is packaged in a lipid membrane which also contains proteins (envelope protein and spike protein). The spike is the viral protein that binds to the host cell's main receptor (ACE2).
To do this, the spike must possess a receptor binding domain (RBD). The SARS-CoV-2 spike also has a furin cleavage site. Furin is a ubiquitous enzyme present in many human (and animal) cell types, it allows the cleavage of the spike between its S1 and S2 subunits. The spike is synthesized as an inactive precursor which must be cleaved to ensure membrane fusion. The trimeric spike is cleaved at the S1/S2 site by host proteases during infection. Then the RBD located in S1 recognizes a cellular receptor, ACE2.18

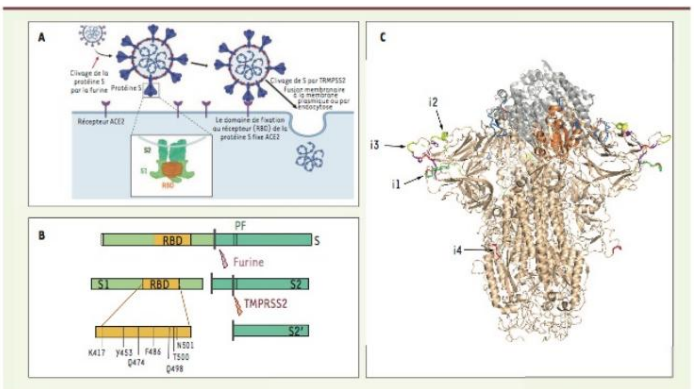


Figure 3. Structure et fonctions de la protéine S (spicule, spike en anglais). A. Représentation schématique de l'infection des cellules par le SARS-CoV-2 après fixation de la protéine S au récepteur ACE2. ***B.*** La protéine S subit deux étapes de maturation par clivage protéolytique (par les protéases furine puis TMPRSS2) nécessaires à son activation et à la libération du peptide de fusion. ***C.*** Structure de la protéine S fixée au récepteur ACE2. La structure de la protéine S de SARS-CoV-2 (en beige) est obtenue grâce au logiciel SWISSMODEL sur la base de la structure 6acc de SARS-CoV (disponible dans Protein Data Bank [PDB]), et alignée sur la structure d'un domaine RBD (en orange) interagissant avec ACE2 (en gris) issue du modèle 6m0j (disponible dans PDB). Les sites d'insertion sont indiqués en couleur. Les résidus sont colorés en fonction de l'ordre de conservation des insertions, en passant du rouge (insertion présente uniquement chez SARS-CoV-2), au jaune, vert, bleu clair puis indigo (insertion présente chez la majorité des sarbécovirus [sous-genre de coronavirus regroupant les virus apparentés à celui du SRAS]).

Figure taken from "Recovering the origins of SARS-CoV-2 in phylogenies coronavirus »
Erwan Sallard, José Halloy, Didier Casane, Jacques van Helden, Etienne Decroly https://www.medecinesciences.org/fr/articles/medsci/pdf/2020/07/msc200195.pdf

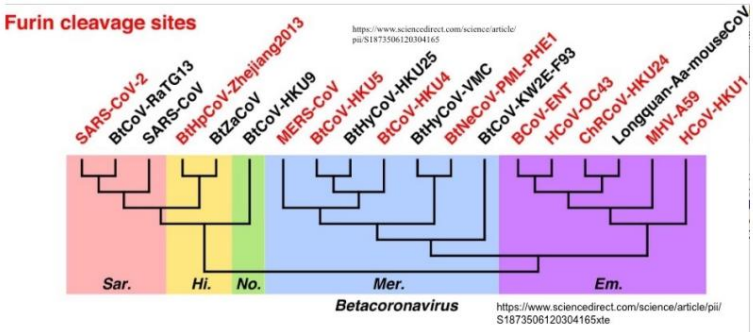
If we compare SARS-CoV-2 with its predecessors (the 2 coronaviruses having caused more localized epidemics, MERS and SARS-CoV of 2003), it is the only one to possess both this furin site and an RBD binding to human ACE2 (MERS binds to another receptor19.

Table 1. The Receptors for the Human Pathogenic Coronaviruses.

https://www.sciencedirect.com/science/article/pii/S200103702030355X

Subfamily	Name	Receptor
alpha-coronavirus	HCoV-229E	aminopeptidase N (APN) [3], [82]
alpha-coronavirus	HCoV-OC43	N-Acetylneuraminic acid (Neu5Ac or NANA) [10], [83]
beta-coronavirus	SARS-CoV-1)	angiotensin converting enzyme 2 (ACE2) [10], [62], [84]
beta-coronavirus	HCoV-NL63	angiotensin converting enzyme 2 (ACE2) [10], [64]
beta-coronavirus	CoV-HKU1	dipeptidyl peptidase 4 (DPP4) [10], [85]
beta-coronavirus	MERS-CoV	dipeptidyl peptidase 4 (DPP4) [10], [86]
beta-coronavirus	SARS-CoV-2	angiotensin converting enzyme 2 (ACE2) [21], [68]

The cellular receptors of the different pathogenic coronaviruses for humans (figure taken from Teng S, Tang Q. ACE2 enhance viral infection or viral infection aggravate the underlying diseases. Comput Struct Biotechnol J. 2020;18:2100-2106. Published 2020 Aug 6 .doi:10.1016/j.csbj.2020.08.002)



Furin cleavage sites naturally occur in coronaviruses. Stem Cell Res. 2020 Dec 9;50:102115. doi: 10.1016/j.scr. 2020.102115. Epub ahead of print. PMID: 33340798; PMCID: PMC7836551.)

The virus can enter the cell in two ways and furin will facilitate these two modes of entry.
The furin site at the S1/S2 junction has been shown for both MERS-CoV spike and SARS-CoV-2 to promote entry into lung cells, and to contribute to viral pathogenesis in animal models of SARS-CoV-220.

The high affinity of the spike binding domain (RBD) for human ACE2 observed from the first isolates of SARS-CoV-2

The affinity of the spike binding domain of SARS-CoV-2 for human ACE2 is higher than that of SARS-CoV21.

Early SARS-CoV-2 isolates were surprisingly well matched to human ACE2, which could explain its rapid transmission. Human ACE2 exhibits the strongest binding interaction, significantly superior to all species proposed as the source of the virus22. A rapid adaptation of SARS-CoV occurred during the SARS epidemic in 2002 and 2003: when SARS-CoV was transmitted from civet cats to humans, the spike gene underwent selection positive, in which mutations in two critical residues (amino acids 479 and 487) of the spike protein increased the binding affinity of the virus to human ACE2 from low to high, transforming it into a pandemic strain, following the spread of the virus by a superspreader in which it could have acquired an increased transmission capacity23.
The 2002 SARS-CoV-1 epidemic could result from several different introductions from wild animals.
24

Other receptors than ACE2

There are undoubtedly other cellular receptors than ACE2 allowing the infection of cells by SARS-CoV-225: the LFA-1 protein (leukocyte function-associated molecule) expressed exclusively in leukocytes would allow the virus to penetrate into T lymphocytes without using ACE2 which is not expressed in these cells; this could explain the lymphopenia observed in Covid-19 patients. This receptor is also used by HIV to bind to CD426 lymphocytes. An article proposed it in April 2020, it was withdrawn by the authors under scientific pressure27 : it was not good to mention a functionality close to HIV for SARS-CoV-2!

SARS-CoV-2 (like SARS-CoV, HIV-1 and Ebola virus) also uses receptors DC-SIGN (dentritic cell-specific ICAM-grabbing non integrin) to penetrate cells through spike glycans28). Cultured human respiratory cells are also infected by DC binding SIGN.

DC-SIGN receptors are expressed on monocytes and macrophages derived from dendritic cells, LAF-1 on leukocytes (including T cells and DC). DC-SIGNs are able to bind

to the gp120 glycoprotein of the HIV-1 envelope and to increase the infection of T cells. These 2 types of receptors are different but the overall architecture of their binding domains could be similar29.

HIV homology sequences

Montagnier-Perez hypothesis

They discovered30 in SARS-CoV-2 a 225 nucleotide sequence that is absent from all coronaviruses (except RaTG13 which is certainly a laboratory construct). This sequence contains four specific regions of HIV (HIV1 and HIV2). Concerning HIV1, the sequences are those of a strain having been used to create an anti-HIV vaccine candidate.

HIV Gp120 sequences bind to DC-SIGN31 and all 3 are found at spike binding sites of SARS-CoV-2. The 4th sequence has homology with the HIV gag protein and concerns the furin site.

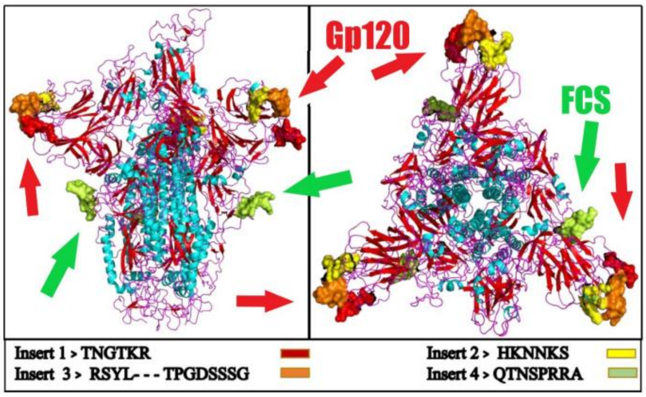


Figure 3. Modelled homo-trimer spike glycoprotein of 2019-nCoV virus. The inserts from HIV envelop protein are shown with colored beads, present at the binding site of the protein.

Figure taken from the retracted article by Pradhan *et al. :* the homology sequences with HIV are located on the binding sites of the spike.

Before the first Montaginer Perez publication, an Indian article (January 31, 2020) withdrawn from publication following criticism also showed the existence of these HIV homology sequences on the binding sites of the spike of SARS-CoV-232 . The authors voluntarily withdrew the article in the face of the attacks they suffered; then they checked their work and tried to repost but wiped the refusal of all the journals contacted; however their work was later confirmed33

In summary , the Covid-19 virus has several molecular characteristics that explain its ability to infect humans and its pathogenicity: the ACE2 receptor binding domain (RBD), the furin cleavage site, its ability to bind to DC-SIGN and LAF-1 receptors and homology sequences with HIV (the HIV gp120 sequence binding to DC-SIGN is capable of reactivating a latent HIV provirus. Since these homologous sequences are found in SARS-CoV- 2 they could be responsible for the reactivation of AIDS in infected people34.

The different hypotheses on the origin

An article in French by 3 internationally renowned Marseille virologists (published in April 2021 in Virologie) 35 reviews the arguments in favor of a natural origin. The research programs conducted at the Wuhan Institute of Virology (WIV) by EHA and co-funded by the NIH are entirely compatible with the hypothesis of a laboratory accident.

According to them, the WHO report on the investigation in China (March 2021) proposes 4 hypotheses: 2 concern an animal origin, one the arrival in China via frozen foods or the cold chain, and finally an accident. of laboratory.

“These working hypotheses are classified from highly probable to very improbable by the commission without the rational bases for this classification being substantiated and while the escapes of laboratory viruses have been documented in the literature”.

The authors point out that viruses can only pass from bats to humans after a series of events: the ability to bind to human ACE2, to increase human transmissibility by acquiring a proteolytic cleavage site by furin, a ubiquitous cellular protease; without these 2 events the passage from bats to humans is possible but extremely rare and does not cause an epidemic (it occurs by "spillover", overflow, when an individual is infected by very large quantities of virus as for the miners of the mine of Moijang 36).

No animal having played the role of intermediate host could be identified, this invalidates the high probability affirmed by the WHO of the 2 hypotheses of animal origin. The frozen meat hypothesis has no scientific basis and does not explain the origin of the virus.

The main arguments in favor of the synthetic origin

Virus escapes from laboratories have been documented in the literature. For SARS-CoV, there are 3 documented cases of escape of this virus from P3 and P4 security laboratories (Singapore, Taiwan and China)37. This also applies to other infectious agents 38.
The H1N1 virus emerged twice from a laboratory: in 1977 and in 2009 (it was the cause of the 2009/2010 pandemic, probably due to a badly inactivated vaccine according to Furmanski 39.

As we will see, the gain-of-function research programs conducted at the WIV and co-funded by the NIH are compatible with the artificial origin of the virus. Indeed, these gains in function concern the acquisition of the ability for the virus to bind to human ACE2 in order to anticipate the emergence of a potentially pandemic virus and to develop in advance vaccine strategies to answer. Numerous experiments involving the insertion of sites rich in basic amino acids at the S1/S2 junction have already been carried out in order to potentiate infection by CoVs ("furin sites") ("Virology" and see these experiments below).
Unusual characteristics of SARS-CoV-2 suggest manufacture in the laboratory followed by adaptation to humans by passage in humanized mice, ie genetically transformed to express human ACE2, its cellular receptor40.

Association of ancestral SARS-CoV-2 sequences with sequences from lab-grown cells: SARS-CoV-2 single-variant host genomes infiltrated Antarctic soil metagenomic sequencing data

Researchers have found sequences of an ancestral SARS-CoV-2 in soil samples collected in December 2019 in Antarctica. They showed that these sequences did not come from Antarctica but that they had contaminated the sequencing device in Shanghai41. Sequences of SARS-CoV-2 isolated from the first Chinese patients were deleted from databases and found on the internet by Jesse Bloom42
There are strong similarities between these 2 groups of sequences, some of which contained three key mutations: C8782T, C18060T, and T28144C. A virus with these three mutations compared to Wuhan-Hu-1 is one of two plausible progenitors of all currently known human SARSCoV2s. In the same sample are found sequences that may come from the hosts that hosted these primitive SARS-CoV-2: human mitochondria, CHO cells (chinese hamster ovaries) and Vero. The fact that these primitive sequences are close to those of RaTG13, that they have been found associated with sequences of laboratory cells that could have been used to cultivate SARS-CoV-2 feeds the hypothesis of a virus that escaped from a laboratory.

Arguments against the natural origin

For coronavirologists43 it is not possible to decide between natural and synthetic origin.
Bioinformatics analysis of the SARS-CoV-2 genome shows a codon usage bias suggesting possible genetic manipulation: if the virus had appeared naturally, the proportion of each codon in the genome would have been different because in the natural virus RNAs the distribution of the codons remains in fairly stable relative proportions (note 18).

This is confirmed by Ralph Baric (professor at UNC -University of North Carolina- and part of the PREDICT and DEFUSE projects), who affirms that in 2019 it was possible to create a chimera virus without leaving traces, unlike 2Ǫ1544. Ralph Baric, author of the 2015 chimera virus45 stated:

- You can create a virus without leaving a trace. The answers you seek, however, can only be found in the Wuhan lab archives."
- "In the chimera that we did in America in 2015 with the SARS virus, with Professor Zheng li Shi from the Wuhan Institute of Virology, we had left signature mutations, so it was understood that it was the result of genetic engineering. But, otherwise, there is no way to distinguish a natural virus from a virus made in a laboratory".

The virus genome sequences can be downloaded but the Wuhan databases have disappeared.
As of June 2020, the entire page has been removed from the web. The data was inaccessible as of September 12, 2019.

According to E Decroly et al. (note 12), Zheng-Li Shi proposed that the virus comes from a bat virus, RaTG13, an isolate of which was collected in 2013 and stored in the Wuhan laboratory. RaTG13 was only sequenced in 2017-2018 and published in February 2020. This hypothesis seems completely invented *a posteriori* because the name of this strain was changed without explanation (BtCoV/4991 initially), and the RBD of RaTG13 has only 70% homology with that of SARS-CoV-2. There is some doubt about the veracity of the RaTG13 sequence because if it had really been collected from bat feces, we would expect to find RNA from bats and bacteria there, which is not the case. case.

In addition RaTG13 has an RBD (receptor binding domain) capable of binding to human ACE2 (like SARS-CoV-1) which must have been obvious to Me Shi if she had it really isolated in 2013 and sequenced in 2017-18: she would not have waited for the start of the SARS-CoV-2 pandemic to publish the

RaTG13 sequence. The report of synonymous and non-synonymous mutations and their distribution as well as the sequence of the E protein of SARS-CoV-2 also sign an artificial synthesis of this genome 46.
The natural origin is based on the spillover hypothesis (overflow: contamination of a species other than that to which a virus is adapted by a massive inoculum effect, this occurred in a Chinese mine infested with bats but the miners did not transmit the disease to anyone). Regarding SARS CoV-2, this seems unlikely because it is poorly adapted to bats: it does not replicate in their kidney or lung cells, this is against a spill-over from of the bat.

The pangolin hypothesis raises many questions: the recombination would have occurred between a pangolin virus and a bat virus, however, no intermediate virus that would result from this recombination has been identified to date.

How did one come to suspect artificial origin more than strongly?

January 2020:

A Chinese bioinformatics team publishes in Chinese in January 2020: this is the first mention of the furine47 site. On January 31, 2020 an article withdrawn since and quoted above also alluded to it (Pradhan *et* al., 2020)

Researchers from two teams from Aix Marseille University, the CNRS and the University of Montreal have identified the furin site in the sequence of the "Spike" protein of 2019-nCoV (the first name given to SARS CoV-2) They hypothesize that this motif is an important factor in the emergence or pathogenicity of the virus (the insertion of a multibasic motif at the cleavage site of the HA hemagglutinin of the H5N1 virus has probably been associated with the hypervirulence of the virus during the Hong Kong outbreak in 1997)48.
The common cold coronaviruses HCoV-OC43 and MERS possess the furin cleavage site in the spike.
SARS-CoV-2 has 12 additional nucleotides coding for a PRRAR polybasic site (spike amino acids 680 to 684: ProlineArgininеArgininеAlanineArginine) corresponding to a furin cleavage site which represents a gain of function for this virus compared to the others beta-coronavirus, allowing it to spread through the human population. This was confirmed on February 18, 2020 by an American-French team49.

February 2020:

WIV's Zheng-Li Shi publishes spike S1 subunit amino acid sequence alignment of SARS-CoV-2 with the other coronaviruses in Nature50. The comparison stops at amino acid 675, just before the newly appeared furin site. Zheng-Li Shi claims that the only significant changes in the sequence of the new virus compared to other known coronaviruses are found elsewhere than at the furin site!
This allows him to conclude that SARS-CoV-2 comes from RaTG13 because it is very close to it. Moreover, according to the addendum on RaTG13 November 2020: the complete genome of RaTG13 would have been sequenced in 2018 (Me Shi would not have seen there the RBD able to bind to human ACE2, and did not publish it).
These two "oversights" amount to 2 confessions on the part of Madame Shi: we cannot expect her to publicly acknowledge having inserted this furin site! I don't like scientific metaphors but it's as if a police inspector sent to the scene of a crime overlooked the presence of a bloody knife!
I would personally add that from an evolutionary point of view, the probability that these 2 characteristic mutations (RBD binding to human ACE2 and furin site) appeared by chance at the same time on a virus is negligible or even nil: in indeed there is no natural selection pressure for a virus perfectly adapted to a wild animal to mutate and jump to humans with such efficiency (the same cannot be said of the viruses of farm animals which make animal-human return trips). This argument is also given by Segreto et al. 202151. The appearance of the furin site is not accompanied by other point mutations in the sequence (compared to previous natural viruses), which would have been expected during natural evolution.

Furin site insertion and ACE2-binding RBD experiments have been conducted since 2004: Between 2004 and 2015, most of these experiments were conducted by teams led by Ralph Baric and Zheng-Li Shi who are stakeholder in the DEFUSE project: they concern the furin site, the link to ACE2 coronaviruses as well as binding to DC-SIGNs, presumably to anticipate a virus capable of overcoming the transmissibility failure of SARS-CoV.

2004 - A non-coronavirus furin site insertion patent is filed to allow a vaccine candidate pseudovirus to enter all mammalian cells, then these viruses would self-destruct inside the cells52.

2008 - Ralph Baric constructs a virus chimera with a modified spike protein binding domain to explore the emergence of future human pathogens; this work had just been made possible by new complementary DNA synthesis technologies. It already concerned the RBD (in S1) of the spike and

regions of S2. It was recalled that polioviruses and the 1918 influenza virus had already been reconstructed with these techniques as well as retroviruses. Work funded by NIAID.53
2009: A study proposes 2 possible locations for the insertion of a furin cleavage site in the spike of coronaviruses, one of them concerns the sequence at the S1-S2 junction (amino acids 664 to 671). The inclusion of these sites greatly increases the infectivity of coronavirus54.
Peter Daszak, co-author with Zheng-Li Shi of an article from 201355, justifies these experiments to prepare for future pandemics.
2014 - Mrs. Zheng-Li Shi, director of the Center for Emerging Infectious Diseases of the WIV, received more than 1.2 million dollars from the US government between 2014 and 201956. She created with Ralph Baric57 a chimeric virus by inserting the gene of the spike protein from a wild bat virus into the genome of a SARS virus that has been adapted to grow in mice and mimic human disease.
2015: Publication in Nature Medicine58 of an article describing the creation of a chimeric virus by reverse genetics : a spike protein of a virus naturally possessing the ability to bind to human ACE2 is characterized in a bat virus found in China, it is inserted into a virus “skeleton” adapted to mice. The chimeric virus can reproduce SARS disease in mice and is capable of infecting human cells in culture. R Baric raises the question of the danger of this type of experiment: the risk of generating more dangerous pathogens must be weighed against the potential to prepare future pandemics.

Scientists (including one from the Institut Pasteur) warn of the danger of these experiments.
A patent that describes the modifications introduced in these chimeric viruses is taken in 2015 by Ralp Baric. He relates to a chimeric coronavirus spike protein modified for the RBD and the fusion domain; the furin site does not match that of SARS-CoV-259. 2017: the Shi-Daszak group publishes the creation of 8 chimeras from a virus collected from bats and from different RBDs of SARSr (viruses close to SARS) (EHA-WIV) 60. The need for furin for the he adaptation of coronaviruses to new species had been known to R Baric since 201861. The pseudoviruses used in these experiments were plasmids containing the envelope-defective HIV-1 genome.

The furin site of SARS-CoV-2 has the same sequence as EnaC-ÿ (ÿ subunit of the epithelial sodium channel, a protein essential for airway surface fluid homeostasis, the misregulation of which is associated with respiratory diseases62). This site has been studied at UNC63. It is optimized for arginine codons (CGG): these are the best codons found in humans for this amino acid.

HIV homology sequences

As said previously, despite the strong criticism received by the authors of the first publications on this subject, no one has come to dispute the presence of these homologies on the binding sites of the SARS-CoV-2 spike.
Only SARS-CoV-2 and RaTG13 (which is certainly a laboratory construct) contain these HIV sequences.

The furin site alone is not sufficient for the pathogenesis of SARS-CoV-2: the amino acid sequence
QTQTN upstream is also necessary (work by Menachery, Galveston, Texas, 2021, QTQTN motif upstream of the furin cleavage site plays key role in SARS-CoV-2 infection and pathogenesis
Michelle N. Vu, Kumari G. Lokugamage, Jessica A. Plante, Dionna Scharton, Bryan A. Johnson, Stephanea Sotcheff, Daniele M. Swetnam, Craig Schindewolf, R. Elias Alvarado, Patricia A. Crocquet-Valdes, Kari Debbink, Scott C. Weaver, David H. Walker, Andrew L. Routh, Kenneth S. Plante, Vine and D. Menachery
bioRxiv 2021.12.15.472450; doi: https://doi.org/10.1101/2021.12.15.472450)
On the furin site of SARS-CoV-2 (nucleotides 23548 to 23771, amino acids 677 to 686 – QTNSPRRARS), we note that the QTNS sequence is derived from HIV and is located adjacent to the sequence of the PRRAR furin site.
The corresponding HIV-1 Gag site is QTNS-silmqrsnfk-PRRA which is found to be identical at amino acids 366-384 to the gag protein cleavage site of an Indian variant of HIV.

According to Sallard *et* al., the sequences of HIV may have arisen by natural evolution (“sequences sharing the same insertion appear grouped in the phylogenetic tree, suggesting a distinct origin for each insertion”) and indeed one can follow the evolution of the peptide sequences here:

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Loop 1            TNGTKR
SARS-CoV-2  HVSG  TNGTKR FDNP
BANAL-52    HVSG  TNGIKR FDNP
RaTG13      HVSG  TNGIKR FDNP
GX_P4L      NYQG  ...FKK FDNP
MP789       TKTN  S.AEKR VDNP


Loop 2            HKNNKS
SARS-CoV-2  GVYY  HKNNKS WMES
BANAL-52    GVYY  HRNNKS WMES
RaTG13      GVYY  HKNNKS WMES
GX_P4L      GVYY  HNNNKT WVEN
MP789       SGYY  H.NNKT WSTR


Loop 3            RSYLTPGDSSSG
SARS-CoV-2  LALH  RSYLTPGDSSSG WTAG
BANAL-52    LALH  RSYLTPGDSSSG WTAG
RaTG13      LALH  RSYLTPGDSSSG WTAG
GX_P4L      LALH  RSYLTPGNLESG WTTG
```

The Banal-52 strain was isolated in 2020 in Laos and does not contain the furin site (Temmam, S., Vongphayloth, K., Salazar, EB et al. Bat coronaviruses related to SARS-CoV-2 and infectious for human cells Nature (2022).https://doi.org/10.1038/s41586-022-04532-4)
These homologies may have appeared by natural evolution and have been optimized by Daszak and Baric (DEFUSE project).

Gains of Function (GoF) Debate

Gain-of-function (GoF) experiments consist of increasing the ability of an infectious agent to cause disease (through increased pathogenicity and/or transmissibility).

2011-2012: Great debate on GoFs

Following manipulations in animals on the H5N1 virus to anticipate its evolution allowing it to be transmitted from human to human, attempts by American laboratories to publish their results have launched a great debate on so-called gain-of-function experiments (GoF) with pathogens with pandemic potential64. A moratorium on GoF experiments on influenza viruses, MERS and SARS was decided on October 17, 2014 in the USA65 : “No new funding from the United States government will be granted for research projects on the gain of function that can reasonably be expected to *confer attributes on influenza, MERS, or SARS viruses such that the virus would have increased pathogenicity and/ or transmissibility in mammals via the respiratory route.* The pause in research funding does not apply to the characterization or testing of naturally occurring influenza, MERS and SARS viruses, unless it is reasonably expected. that testing increases transmissibility and/or pathogenicity. »

December 19, 2017: Moratorium on GoFs lifted by Francis Collins, Director of NIH66. The Secretary to the Department of Health, HHS (Health and Human Services) had resigned and the position remained vacant until January 218; the NIH and A Fauci took the opportunity to quietly relaunch GoF funding to the great astonishment of the scientific community.

Anthony Fauci has been director of the NIAID National Institute of Allergy and Infectious Diseases since 1984, he has worked at the NIH since 1968. He has defended gain-of-function research: he asserted in 2012 that "the risk-benefit ratio of such research clearly leans in favor of society"67, for him moreover, "Nature itself is the most dangerous of bioterrorists. ".
Senators Ron Johnson and Rand Paul were on this Committee on Homeland Security and Governmental Affairs; they returned to the charge in 2020 and 2021 against A Fauci over NIH-funded coronavirus GOFs. We are struck by the similarity of the experiments carried out on the influenza virus and on coronaviruses.

Peter Daszak, head of the **Eco Health Alliance,** funds the GoFs (EHA, an NGO with an Orwellian profession of faith68: "EcoHealth Alliance: Standing Between You and the Next Pandemic" the profession of faith was recently modified: the previous one was "" Working for a world without pandemics"»69). He was part of the WHO commission of inquiry chaired by Peter Embarek, responsible for investigating the Wuhan laboratory.
This commission concluded that it was extremely unlikely that the virus had escaped from the laboratory. We can measure the extent of the collusion of interests of P Daszak, when we know that he was a contractor, collaborator and co-author of work carried out at the WIV on the construction and analysis of new chimeric coronaviruses.
In October 2017 the NIH/NIAID had received the report of a visit to the P4 laboratory in Wuhan by one of its agents on site, this document is mentioned in the emails revealed by FOIA but he did not been published70.
In an interview published on the internet71 P Daszak explains to us the experiments carried out by EHA "you can manipulate the virus in the laboratory, the spike protein is responsible for the virus's ability to infect an animal, you can modify the sequence of the spike protein (construct a protein), that's what we do with Ralph Baric, we insert the sequence of this protein into another virus. We are trying to develop a vaccine against this new virus that we are building to anticipate a pandemic”
According to Science72, the NIH funded $3.7 million in EHA for 5 years (part of the sum was sent to the WIV).

The EHA collaboration with the WIV dates back to 2004, Peter Daszak wrote 18 publications with Zheng-Li Shi.
Finally in an article published in 201673, it is clear that GoFs have been carried out; the authors also state that the research was supported by NIAID under grants U19AI109761 and U19AI107810, which together total $41.7 million.

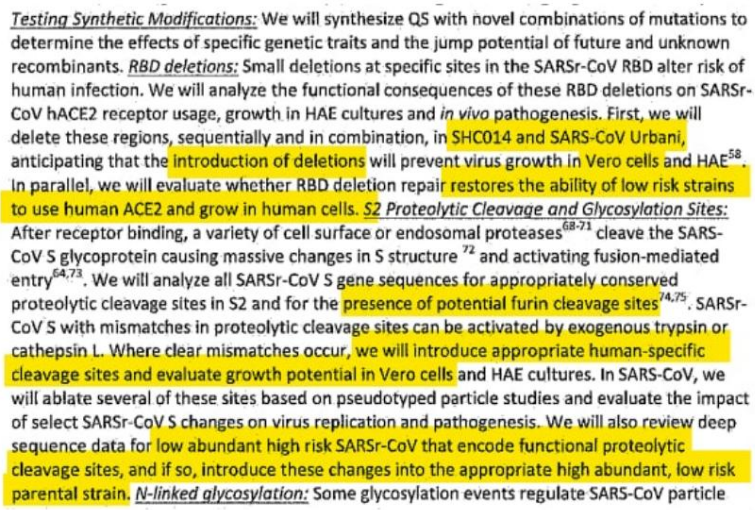
This document makes it clear that NIAID spent this amount on GoF research, with the goal of determining how bat coronaviruses can be made more pathogenic to humans, and that this research continued after the release. implementation of the 2014 moratorium on this type of financing.

The latest declassified documents: aborted collaboration between EHA and the US Army

DARPA-DEFUSE

To protect its soldiers sent on expeditions from the dangers of future emerging viruses, the US Army, through the DARPA (Defense Advanced Research Projects Agency) issued a call for tenders in 201874
for research to anticipate the emergence of future coronaviruses with pandemic potential. This call for tenders concerns the development of models allowing this prediction, the verification of the validity of these models by *in vivo* experiments on different animal species evaluating the capacity of the modeled viruses to jump

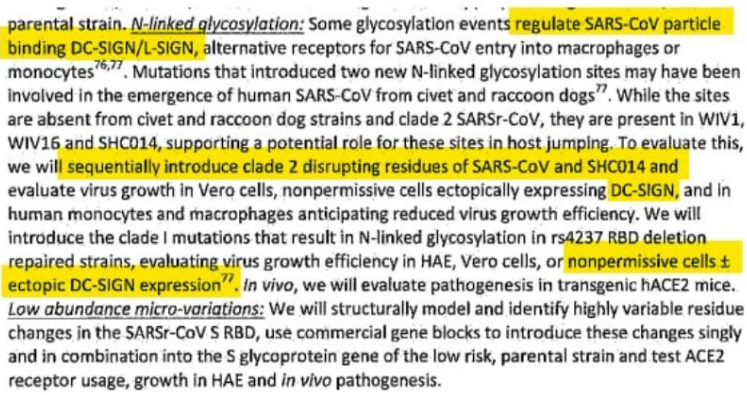
from one species to another and finally the means of preventing the spread of these viruses from their animal reservoir, which are bats (by eliminating this virus).
EHA responded to this call for tenders but its DEFUSE75 project was rejected by DARPA for the following reasons, including the GoF: EHA proposed to build a virus with the 2 characteristics necessary to cause a pandemic: a binding domain of the spike (RBD) capable of binding to the human ACE2 receptor and a cleavage site by furin (human ubiquitous enzyme). However, DARPA pointed out that some proposed experiments could be funded, so it cannot be said that DARPA did not fund EHA76



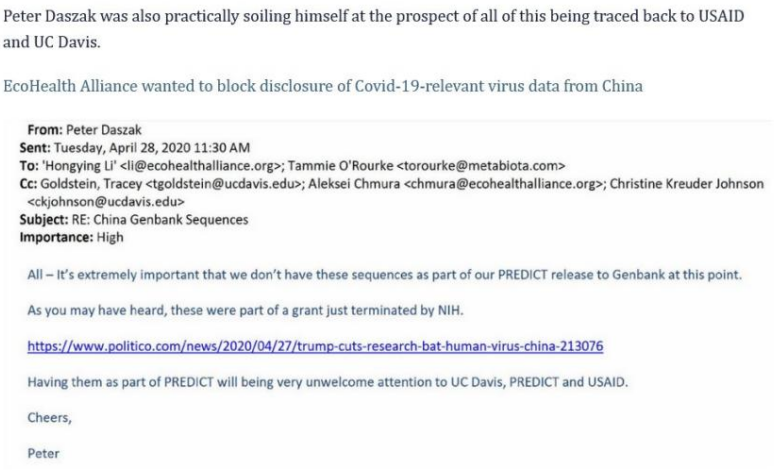
Testing Synthetic Modifications: We will synthesize QS with novel combinations of mutations to determine the effects of specific genetic traits and the jump potential of future and unknown recombinants. *RBD deletions;* Small deletions at specific sites in the SARSr-CoV RBD alter risk of human infection. We will analyze the functional consequences of these RBD deletions on SARSr-CoV hACE2 receptor usage, growth in HAE cultures and *in vivo* pathogenesis. First, we will delete these regions, sequentially and in combination, in SHC014 and SARS-CoV Urbani, anticipating that the introduction of deletions will prevent virus growth in Vero cells and HAE[58]. In parallel, we will evaluate whether RBD deletion repair restores the ability of low risk strains to use human ACE2 and grow in human cells. *S2 Proteolytic Cleavage and Glycosylation Sites:* After receptor binding, a variety of cell surface or endosomal proteases[68-71] cleave the SARS-CoV S glycoprotein causing massive changes in S structure [72] and activating fusion-mediated entry[64,73]. We will analyze all SARSr-CoV S gene sequences for appropriately conserved proteolytic cleavage sites in S2 and for the presence of potential furin cleavage sites[74,75]. SARSr-CoV S with mismatches in proteolytic cleavage sites can be activated by exogenous trypsin or cathepsin L. Where clear mismatches occur, we will introduce appropriate human-specific cleavage sites and evaluate growth potential in Vero cells and HAE cultures. In SARS-CoV, we will ablate several of these sites based on pseudotyped particle studies and evaluate the impact of select SARSr-CoV S changes on virus replication and pathogenesis. We will also review deep sequence data for low abundant high risk SARSr-CoV that encode functional proteolytic cleavage sites, and if so, introduce these changes into the appropriate high abundant, low risk parental strain. *N-linked glycosylation:* Some glycosylation events regulate SARS-CoV particle

EHA proposed to synthesize spike proteins that bind to the human ACE2 receptor and to insert them into the skeletons (genomes) of SARSr-CoV (bat virus close to SARS-CoV), it was also a question of inserting a site of furin cleavage in synthetic viruses (SARS CoVs do not have this site).

On page 11, EHA emphasized the importance of the spike glycosylation sites of the SARS-CoV-1 in binding to DC-SIGN receptors for entry into macrophages and monocytes; EHA proposed to introduce into chimeric viruses the mutations favoring these glycosylations found on strains collected recently from bats (WIV1, WIV16 and SHC014).
These chimeric viruses would be introduced into cells in culture (in particular HAE, human airway epithelial cells) and their pathogenesis in hACE2 transgenic mice will be evaluated.



parental strain. *N-linked glycosylation:* Some glycosylation events regulate SARS-CoV particle binding DC-SIGN/L-SIGN, alternative receptors for SARS-CoV entry into macrophages or monocytes[76,77]. Mutations that introduced two new N-linked glycosylation sites may have been involved in the emergence of human SARS-CoV from civet and raccoon dogs[77]. While the sites are absent from civet and raccoon dog strains and clade 2 SARSr-CoV, they are present in WIV1, WIV16 and SHC014, supporting a potential role for these sites in host jumping. To evaluate this, we will sequentially introduce clade 2 disrupting residues of SARS-CoV and SHC014 and evaluate virus growth in Vero cells, nonpermissive cells ectopically expressing DC-SIGN, and in human monocytes and macrophages anticipating reduced virus growth efficiency. We will introduce the clade I mutations that result in N-linked glycosylation in rs4237 RBD deletion repaired strains, evaluating virus growth efficiency in HAE, Vero cells, or nonpermissive cells ± ectopic DC-SIGN expression[77]. *In vivo*, we will evaluate pathogenesis in transgenic hACE2 mice. *Low abundance micro-variations:* We will structurally model and identify highly variable residue changes in the SARSr-CoV S RBD, use commercial gene blocks to introduce these changes singly and in combination into the S glycoprotein gene of the low risk, parental strain and test ACE2 receptor usage, growth in HAE and *in vivo* pathogenesis.



Peter Daszak was also practically soiling himself at the prospect of all of this being traced back to USAID and UC Davis.

EcoHealth Alliance wanted to block disclosure of Covid-19-relevant virus data from China

From: Peter Daszak
Sent: Tuesday, April 28, 2020 11:30 AM
To: 'Hongying Li' <li@ecohealthalliance.org>; Tammie O'Rourke <torourke@metabiota.com>
Cc: Goldstein, Tracey <tgoldstein@ucdavis.edu>; Aleksei Chmura <chmura@ecohealthalliance.org>; Christine Kreuder Johnson <ckjohnson@ucdavis.edu>
Subject: RE: China Genbank Sequences
Importance: High

All – It's extremely important that we don't have these sequences as part of our PREDICT release to Genbank at this point.

As you may have heard, these were part of a grant just terminated by NIH.

https://www.politico.com/news/2020/04/27/trump-cuts-research-bat-human-virus-china-213076

Having them as part of PREDICT will being very unwelcome attention to UC Davis, PREDICT and USAID.

Cheers,

Peter

In a declassified email from April 2020 from P Daszak we can read this: "it is extremely important that we only give not these sequences as part of the publication of PREDICT in Genbank at this stage. » These are the sequences that underlie the GP120 peptide sequences of SARS-CoV-2. That is why Pradhan only found the peptide sequences on which they were based. : in GenBank or BLAST only the nucleotide sequences of HIV and SARS-CoV-2 are present and not those of the PREDICT project.

According to DARPA these experiments represent a GoF but it is not mentioned in the project, the risks are not assessed, the proposed vaccine administration system poses problems of dosage of quantity delivered, these vaccines would not be able to protect bats against the wide variety of existing and evolving wild viruses because they would not cover not enough epitopes.

It is here that a break in the logic of reasoning occurs. The authors of the DEFUSE project

anticipate the realization of their modeling as if they had mastered "The Time Machine"!

The designers of the EHA project are so certain of having modeled the future pandemic viruses that they plan to vaccinate bats against these viruses that have not yet appeared with precisely these living synthetic viruses!
They therefore consider that these viruses will appear naturally in bats and that it will therefore be necessary to immunize these animals to prevent them from transmitting them to humans.
To check the possibility of vaccinating bats against this future virus, EHA proposes using a live aerosolized virus; why not have tested this possibility with a non-humanized virus or with an inactivated virus or a pseudovirus incapable of replicating?
Not only does the EHA offer prohibited gain-of-function experiments, but it also plans to distribute viruses with pandemic potential by aerosol: we understand DARPA's caution! However, according to the documents declassified thanks to the DRASTIC group (76), the DARPA did not exclude financing certain parts of the project if other financing were found: the refusal related mainly to the amount requested.
This research on coronaviruses at pandemic risk was not an American-Chinese exclusive: in a 2018 thesis defended at the Institut Pasteur Paris, we learn that a furin site was inserted into the spike of HCoV-229E (a coronavirus common cold) but using a pseudovirus with a skeleton of MLV *(Murine Leukemia Virus)* incapable of replicating: it is therefore possible to model the appearance of a virus with pandemic potential without taking the risk of causing a pandemic!77

Collaboration between NIH/NIAID and EHA

The DEFUSE project discussed above is for a DARPA tender, but EHA has a long history of working with the NIH. Funding for coronavirus gain-of-function projects dates back to the early 2000s.

R Baric has worked since the 2000s on the search for anti-SARS vaccines and on the determinants of the pathogenesis of coronaviruses. We find in his CV78 the following contract: *National Institute of Health, Allergy and Infectious diseases. "Reverse Genetics with a Coronavirus Infectious cDNA Construct." 4/1/2001-3/31/005 $1.0 million total costs/yr. RS Baric, PI 25% effort. GM 63228* which led to a publication in 200379. It was already a question of manipulating the SARS-CoV genome in order to study its pathogenic effect and to develop candidate live attenuated vaccines.
The HHS (Health and Human Services) considers that GoF experiments can be authorized when it comes to developing and producing vaccines against a potentially pandemic pathogen80, this document allowed the lifting of the moratorium on GoF established in 2014.
In the DEFUSE project, it is specified that some of the experiments have already been carried out. The details with which the planned experiments are described led certain specialists to suppose that they had been partly carried out. This is confirmed by the reports cited above and disclosed by The Intercept's FOIA (Freedom Of Information Act).

In the current contract between EHA and the NIH/NIAID81 , it reads that EHA and WIVI (Wuhan Institute of Virology) constructed at least 3 chimera viruses using NIH funds.
One of them *(SHC014 WIVI)* is able to generate a viral load more than 10,000 times higher and greater pathogenicity in humanized mice (carriers of human ACE2) compared to the parental strain.

According to the agreement signed with the NIH, EHA should have immediately stopped these GoF experiments (it was planned if a 1 log increase was found, here we are at 3 log) and informed the NIH. This agreement dates from 2016.
NIH Deputy Director Lawrence Tabak (NIH Deputy Director) acknowledges that EHA did not notify the NIH of this 2018-2019 experiment that gave rise to a virus possibly pathogenic to humans.
This confirms that the previous assertions of F Collins (director of the NIH), A. Fauci and L. Tabak are false: the NIH did indeed fund GOF experiments in Wuhan.
The NIH was made aware of this data in March 2018 and again in November 2018 and did not respond.
Peter Daszak, the president of EHA affirms in 2022 that it is possible that the virus comes from a laboratory leak, he admits not knowing what Ralph Baric really did concerning the furin site; he also acknowledges that he did not communicate to the NIH the results of the experiments on humanized mice which showed that they had obtained much more pathogenic strains following the GoFs, but he then asserts that the NIH was aware of the experiments on humanized mice conducted in Wuhan.

The funding also involved experiments on MERS viruses modified to infect cells humans and humanized mice (the natural MERS has the furin site but not the receptor for human ACE2, the lethality of this virus is 30%). NIH/NIAID R01 grant AI110964 involved mutating MERS viruses like NeoCoV and PDF-2180 so that they can infect human cells: replacing the RBD of MERS with the RBD of different HKU4-related strains; virus recovery

chimeras, infection of human cells from different tissues (lung, liver, intestine, kidney), replication in cells possessing the DPP4 receptor: the results suggest a potential risk of infection of humans by these viruses. Close relatives of MERS-CoV in bats use ACE2 as a functional receptor83.

The rest of the results have not been published: did the mutant viruses prove to be too dangerous?, or, after infecting human cells, did the researchers give up infecting humanized mice with a potentially pandemic virus once once the emergence of SARS-CoV-2 is known? Experiments have also been carried out on a porcine virus, SADS-CoV, responsible for fatal diarrhea in farms in China; this virus is capable of infecting human cells in the laboratory according to these documents.
WIV researchers continued to work on MERS2 and published GoF studies in early 2022, but these were conducted in a secure manner since they involve pseudoviruses and not replicative viruses84 : this is VSV-dG (VSV-based rhabdoviral pseudotyping system.
What would have happened if SARS-CoV-2 had not emerged and GoFs with MERS2 had been conducted in humanized mice or with bat vaccines?
This contract is still ongoing: in November 2018, EHA requests a grant extension from the NIH which is accepted for 5 years in June 2018 ($581,646 in 2018 and $661,980 in 2019, July 24), suspended on April 24 2020, in 2020, new funding excluding China is granted in June 2021 ($1.5 million).
This involved, among other things, characterizing the binding domains of the spike and experimenting in vivo with their influence on animal/human transmission.85

We can assume that as early as 2017 this research on chimeric viruses was well advanced since on January 10, 2017, Anthony Fauci announced that there would be a surprise epidemic during Donald Trump's mandate. The Center for Global Health Science and Security at Georgetown University Medical Center (GU GHSS), in partnership with the Harvard Global Health Institute (HGHI), had brought together thinkers from all sectors to listen, learn and discuss how the next presidential administration can contribute to pandemic preparedness, global health security, and national preparedness and resilience.86

NIH collaboration with Moderna

Moderna received $25 million in funding from DARPA in 2013 to develop mRNAs capable of being rapidly deployed in the event of the emergence of a new pathogen87 . In total, in 2015, Moderna had negotiated $450 million in funding (part of which came from DARPA) for its mRNA research concerning in particular mRNA vaccines against Ebola, RSV and other (unspecified) viruses88.

In 2017, S Bancel, CEO of Moderna, decided to redirect the company's research towards vaccines, following safety problems with mRNA therapies intended for rare diseases (low doses were ineffective and high doses too toxic).). Bancel predicted that Moderna could consider having taken off within 5 years (so in 2022)89.

In June 2018, the NIH Vaccine Research Center (VRC) expanded its existing partnership with Moderna to include large-scale research on a pan-coronavirus (CoV) vaccine platform see DRASTIC90.

In **2019,** a Technology Transfer and Profit Sharing Agreement between NIAID and Moderna was amended91. It is a confidential 153-page document that outlines amendments to a collaborative agreement between NIAID and Moderna, signed beginning in 2015; many passages are crossed out.
Previously, Moderna and NIAID had been working on mRNA vaccines for a long time but not on coronaviruses.

It was also a question of membrane proteins stabilized in prefusion configuration, but the furin site only appeared in 2019 (June) as well as the vaccines concerning MERS (April 2019).
Why was the above amendment signed in April 2019 to include MERS trials in the Moderna-NIAID agreement?

The Moderna92 patent

Amabati *et al.* found 100% homology between the 12 nucleotide sequence of the furin site of SARS CoV-2 and a sequence deposited in a Moderna patent of 201693.
However this sequence has not been claimed, it is part of the sequences that can be used in the future (this type of sequence was neither invented nor owned by the patent applicant)
This sequence corresponds (after codon optimization) to a human DNA mismatch repair protein, MSH3.
Optimization for human expression likely has applications in cancers with deficiencies in mismatch repair.

Moderna worked on cancer mRNAs before developing mRNA vaccines.

The authors conclude that the presence in SARS-CoV-2 of a 19-nucleotide RNA sequence encoding a furin site at amino acid 681 of its spike protein with 100% identity with reverse complement of a proprietary MSH3 mRNA sequence is highly unusual. Potential explanations for this correlation need further investigation.

How to link all these gain-of-function experiences together: the key man seems to be Ralph Baric whose CV may shed some light94

R Baric received funding in 2005 to research live attenuated vaccine candidates against SARS-CoV and in 2008 for research on a mucosal HIV vaccine using a common cold coronavirus as a vector. This could explain the SARS-CoV-2 chimera that contains the HIV sequences.

SARS-CoV-2 could have been designed as an attenuated vaccine against HIV or as it is described in the DEFUSE project as a model virus with pandemic potential or even as an attenuated vaccine against all coronaviruses.

Other research on attenuated anti-HIV vaccines goes in this direction.

Live attenuated HIV vaccine (LAV) trials have been conducted in China with an attenuated influenza virus vector95.

Is the pandemic caused by SARS-CoV-2 the result of vaccine research gone wrong?

SARS-CoV-2 has certain characteristics that could have been developed to design a live attenuated vaccine: this type of virus must indeed infect a host with a pathogenicity and a capacity for replication lower than the wild strain that it must combat, while retaining its immunogenicity.

The immune response is mediated by interferons and SARS-CoV-2 has a particular sensitivity to interferons ÿ and ÿ96. Several viral proteins involved in IFN signaling in SARS-CoV-2 appear to be affected by attenuation, such as NSP3, ORF3b and ORF697.

Optimization and de-optimization of codons

Synonymous mutations have been used in the past as a virus mitigation strategy through codon de-optimization.[98]

Comparing RaTG13 with SARS-CoV-2, we clearly observe an accumulation of synonymous mutations in the spike around the furin site: the number of CpG dinucleotides of SARS-CoV-2 is significantly lower than in SARS-CoV or MERS and can also indicate attenuation. On the other hand, the accumulation of CpG in the region of the furin site could direct an immune response towards S in an otherwise attenuated virus.[99]

Recombination resistance is a strategy for the development of live vaccine candidates, as described by R Baric in 2018.[100] A LAV should also not mutate easily and SARS2 appears to be quite resistant to mutations. This characteristic could have been obtained by selecting strains with an RNA-dependent RNA polymerase (RdRp) with improved fidelity.[101]

Researchers with past HIV experience have developed a Covid-19 vaccine candidate based on the gag protein

They point out that the spike of SARS-CoV-2 is very different from all other SARS: it carries an additional charge that greatly enhances its interaction with the DC-SIGN receptor which can mediate endocytosis on its own (without the intervention of the 'ACE2), which may explain the clinical evidence for its infectivity and pathogenicity.102

This research on attenuated vaccines seems to continue:

Ralph Baric's team synthesized and inoculated mice with mutant SARS-CoV-2.[103]

These viruses more pathogenic for mice were obtained by serial passage in mice; they would have an attenuated profile in humans. These more pathogenic viruses have been tested as a vaccine with a platform using pseudoviruses (virus replicon particle (VRP)104

The same teams (Menachery Galveston, Texas) which participated in the gain-of-function experiments continue to manufacture viruses modified from SARS-CoV-2 and infect human cells, mice and hamsters with these viruses and this in the aim of making a vaccine with an attenuated virus.[105]

The same goes for R Baric in 2020.[106]

This team has published the "recipe" for manufacturing SARS-CoV-2 by reverse genetics with a view to studying live attenuated vaccines, to facilitate serodiagnosis, the evaluation of vaccines and the screening of antivirals107.

Some researchers have clustered escape mutations on synthetic spikes embedded in pseudoviruses.[108]

This kind of experiments could be the origin of the Omicron variant

There are different hypotheses, including the emergence in an immunocompromised patient with HIV in South Africa. [109] The virus would have persisted for a long time until it acquired enough mutations to allow it to escape the immunity of surrounding populations.

Murine origin and synthetic origin of Omicron

According to the team of F Balloux and L van Dorp110, minimal adaptation was required for human-to-animal spread and subsequent transmission in mink and deer, highlighting the "generalist" nature of as a pathogen SARS-CoV-2 easily infecting a large number of mammals.

The authors suggest that omicron appeared in mice (wild or laboratory?). Strain wild-type SARS-CoV-2 cannot infect mice, but some variants are able to.
Omicron is not found in any branch of intermediate human evolution that suggests it evolved in an animal. Omicron has 5 mouse-adapted mutations. rare in clinical samples (The Q493 and Q498 mutations are rare in clinical samples and Q498 is found in host animals living in sewers.) Omicron must have evolved in an environment other than that of the previous variants: either in an immunocompromised patient or in a host animal.

The 5 mutations (K417, E484, Q493, Q498, and N501) increase the affinity for mACE2, they are found in the IA-501Y-MA-30 strain obtained after 30 IA-501Y passages in mice.
The phylogenetic trees did not show intermediate branches of evolution: the branch is very long and shows a divergence at the beginning of 2020.
Omicron emerged from a most recent common ancestor MRCA virus that was last seen ~April 2020, and has accumulated over 20 new spike protein mutations.
This means that the omicron was evolving at a speed never seen before (3.3 times faster)
Omicron is much more transmissible than delta. We should have seen many "slightly more transmissible" variants going around the world almost as fast as omicron much earlier.111

The N501Y mutation common to 3 VOCs allows the virus to bind to mACE2; this raises the possibility of secondary reservoirs of wild rodents allowing the emergence of new variants.112

Results suggest that Omicron's progenitor jumped from humans to mice, quickly accumulated mutations favorable to infection of this host and then reverted to humans, indicating a cross-species evolutionary trajectory for the Omicron epidemic.
The furin cleavage site in SARS-CoV-2 gained an additional key arginine in the Omicron, a modification that appears to enhance furin processing during the viral life cycle.113

It is possible to adapt SARS-CoV-2 to mice in 10 passages (R Baric). [114]

Towards a SARS-CoV-3?

In November 2021, the CDC which is part of the Department of Health and Human Services (HHS), has amended their Agents and Toxins regulations to add SARS-CoV/SARS-CoV-2 chimeric viruses resulting from any deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids encoding SARS-CoV-2 virulence factors CoV to the HHS Selected Agents and Toxins List.

These regulations apply to biosafety laboratories classified BSL2 and 3.

SARS-CoV virulence factors include, but are not limited to, those involved in inflammasome activation during infection, which could be introduced into SARS-CoV-2 and create a virulence chimeric virus increased. There is a significant potential risk of fusing a virus carrying known virulence factors and a pandemic virus, the chimeric virus obtained will have the transmissibility of SARS-CoV-2 and the pathogenicity of SARS-CoV115.

This is specific enough to suspect that this research is ongoing as of the date of the edit.
regulations: they could lead to the emergence of the more pathogenic SARS-CoV-3!

Recent biopolitical developments

October 2019:

During a meeting concerning influenza vaccines, the speakers (and in particular Anthony Fauci and Margaret Hamburg, foreign secretary of the National Academy of Medicine), half-heartedly offered to circumvent clinical trials of mRNA vaccines and, with the help of a disruptive crisis, bring them to market without the need for ten years of testing116.

February 9, 2021

The WHO mission117 to investigate the origin of the virus in Wuhan gives a press conference: the hypothesis of the virus leaking from a laboratory is considered highly improbable given the absence of a research project involving coronaviruses close to SARS-CoV-2.
The mission chaired by Peter Embarek, Peter Daszak is one of them. February 14, 2021: Interview with Peter Ben Embarek, head of the WHO fact-finding mission in Wuhan on the origin of the virus.
Contrary to what is announced, this investigation made it possible to no longer exclude an artificial origin of the virus, although this hypothesis is described as “very unlikely”: it is said that before this mission this hypothesis was unthinkable.

We also understand that the emergence of the virus well before December 2019 cannot be ruled out: the Chinese have collected 72,000 cases of flu-like illnesses which appeared during 2019 and which could have been due to Covid-19, but only 92 cases were selected for serological testing and only 67 cases tested negative for SARS-CoV-2. We do not know precisely enough how the Chinese went from 72,000 to 92 cases and on what criteria: according to P Embarek, these 72,000 suspected Covid cases should be re-examined.
In short, nothing is excluded! [118]

Letter from the US Congress, January 11, 2022

Two American deputies address a request to the secretary of the Department of Health in Washington119: Anthony Fauci's emails made public raise the question of whether he was aware of the possibility of the artificial origin of the virus and its escape from the Wuhan laboratory (WIV). Dr. Fauci knew that EHA failed to submit its 2019 report on NIH/NIAID-funded experiments: lawmakers assume this was to hide gain-of-function experiments on deadly, infectious novel coronaviruses.

They suspect Dr. Fauci of having had an article published in Nature Medicine about the origin of the virus in February 2020120 modified : the authors of this article would have concluded that the laboratory leaked.

In these emails we read that virologists do not believe in the possibility of the natural and simultaneous appearance of 12 nucleotides coding for the 4 amino acids of the furin site and this without any modification of the other amino acids of S2.

February 2022 : the 3 French virologists already mentioned ask for a moratorium on gain-of-function experiments concerning viruses with pandemic potential, on gene drive projects and on self-disseminating vaccines (Gene drive, Self-disseminating vaccines, Chimeric viruses... sorcerer's apprentices of the genome, Bruno Canard, Etienne Decroly & Jacques Van Helden, Le Monde Diplomatique, February 2022).

In the USA, Senator Mike Braun will file an amendment on April 4, 2022 to ask HHS to publication of all documents relating to the WIV and the origin of Covid-19. [121]
During a session in March 2022, Senator Susan Collins said that leaking labs is the most likely option; Senator Rand Paul spoke of the gains in office carried out by NIH in Wuhan despite the moratorium which would have been circumvented122.

On March 15, 2022, a bipartisan group in the US Senate passed a bill to establish an independent commission of inquiry into the origin of the virus123.

Also in March 2022, 18 European scientists ask the Presidency of the Commission European Union to launch an investigation into the origins of Covid-19 like the independent senatorial investigation in the USA. They also ask for the publication of the documents relating to EU funding of the Wuhan laboratory124.

The Wuhan laboratory has been funded by the EU since 2015125. A lack of communication was deplored by the European Commission, which had to temporarily interrupt payments in 2020126. The EVAg project must, among other goals, make it possible to anticipate the response to emerging viral diseases.

Conclusion

There is therefore a body of strong arguments tending to prove the artificial origin of the virus: it would come from gain-of-function experiments conducted at the WIV under the aegis of the NIH.
To affirm this with certainty, Ralph Baric and Zheng-Li Shi would have to expressly admit it, which seems unlikely!

Yes there have always been conspiracies in history, but conspiracies are not the principle of explanation of history.

And with this pandemic we have moved to the next level: it is the interest of the entire system that is at stake, we are far beyond a plot.

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