The origin of the Covid-19 virus : update April 2022

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The origin of the Covid-19 virus

Abstract

The non-natural origin of SARS-CoV-2 has been raised and discussed for the past 2 years.
It is important to discuss 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 some aspects of the Covid-19 clinic and therapy.
SARS-CoV-2 is the only one among human pathogenic coronaviruses to possess both a furin polybasic cleavage site and a human ACE2 binding site that explain its ability to infect humans and its pathogenicity.
The main argument against the natural origin of the virus is that no animals acting as intermediate hosts could be identified and no close virus from which it could have evolved naturally was found. In favor of an "artificial" or "synthetic" origin are (among other arguments) past experiments with furin site insertion and human ACE2 binding site insertion, as well as projects revealed by recently declassified documents. SARS-CoV-2 also possesses 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 in great detail in EcoHealth Alliance's response to a DARPA request for proposals. GoFs on coronaviruses began to be funded by the NIH in the early 2000s and involved the Wuhan Laboratory (WIV) thereafter. The European Commission also funds the WIV with the Horizon 2020 project (EVAg and EVA Global).
An investigation is underway in the U.S. Senate and senators have stated that a laboratory leak is the most likely option and have referred to the GoFs conducted by the NIH in Wuhan despite the moratorium that would have been circumvented.

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Definitions: natural or unnatural origin

For the past two years the question of the natural or unnatural origin of the Covid-19 virus has been hotly debated.
What do the terms "natural" and "artificial" mean with regard to the origin of a virus?

Natural origin: it is a zoonotic virus capable of infecting humans and causing a pandemic, and therefore having the capacity to be transmitted immediately and very efficiently from human to human.
In the case of MERS (1) and Ebola (2), sporadic epidemics occur through "spillover", but no pandemic. The SARS-CoV epidemic could have started with several introductions from the wild animal, the civet (3) .

Artificial or synthetic origin: this is a virus from the bat that has been cultivated in the laboratory (on cell lines and in animals) and that escapes from the laboratory.
This virus may have undergone a voluntary modification (human intervention to modify its sequence, or even total synthesis from a sequence modified from 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 has seized on these documents and asked the government specific questions.

On March 5, 2022, The Economist ran a headline on two articles that would provide evidence of the natural origin of the virus and its emergence at the Wuhan wildlife market (4) . This article is the latest in a long series that attempt to prove this natural origin but never provide proof. In particular, the paper by Worobey *et al*. (5), dates its investigation to mid-December 2019, while there are many indications that the emergence of the virus was several months earlier (two European serological surveys found a non-negligible seroprevalence as early as November 2019 (6). We will see that a strong pressure was 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 were withdrawn by their authors in order to avoid future troubles!

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 elaborated by Michel Foucault in the late 1970s): it is the specific policy of the population aimed at optimizing its reproduction and productivity. From an economic point of view, the health status 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, Territoire, 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 try to anticipate economic crises with bank stress-tests, they launch research programs to anticipate and prevent emerging virus pandemics: in particular PREDICT of the USAID (US Agency for International Development) and PREEMPT of the DARPA (Defense Advanced Research Projects Agency)(7) and EVAg and EVA of the European Commission, which must, among other goals, allow the anticipation of the response to emerging viral diseases. (8)

Vaccination has long been a means of this health biopolitics.
In 1760, the mathematician Bernoulli already declared: "If inoculation is adopted, it will result in a gain of several thousand people for civil society; even if it is deadly, as it kills

children in the cradle, it is preferable to smallpox, which kills adults who have become useful to society. ..., Bernouilli concluded that, if one neglects the point of view of the individual, "it will always be geometrically true that the interest of Princes is to favor inoculation." (9)

Biopolitics has focused since the 2000s on widespread vaccination. The WHO declared the eradication of smallpox in 1980 after observing the last case in 1977,(10) and in 1978 the CDC set the goal of eliminating measles by 1982. (11) The UN also hopes for 100% vaccination coverage of the world's population by 2030.(12)
In 2018 the Council of the European Union proposes to strengthen cooperation against vaccine-preventable diseases (13) . This involves increasing vaccination coverage (CV), standardizing vaccine schedules in the EU, creating a European vaccination record. Before the Covid-19 pandemic, measles was the spearhead of this policy, with an objective of 95% CV in 2020, thus meeting the WHO's objectives. Experts are concerned in 2021 about the exaggerated importance of the vaccine industry complex, which risks relegating science to second place behind economics.(14)

The first doubts about the origin of the virus

The first doubts appeared at the beginning of 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.
HIV specialists noticed sequence homologies with this virus and the presence of the furin site jumped out at the 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 proposals within the framework of PREDICT (see below) reinforce the hypothesis of a synthetic origin without, however, formally proving it. The DEFUSE project consisted in anticipating a coronavirus pandemic by constructing a dangerous virus that could emerge and at the same time designing the vaccine and therapeutics to fight it. The chimeric virus described in DEFUSE has several decisive molecular characteristics. These same characteristics are found in the SARS-CoV-2 that has actually emerged and they pose a clinical and therapeutic problem, even when the virus is attenuated and has become endemic as in early 2022. In particular, the furin site allows the virus to infect humans and penetrate many organs; moreover, it is a superantigen that causes specific immunopathological effects compared to other human-infecting coronaviruses.(15)
The knowledge of the molecular characteristics of SARS-CoV-2 is therefore important from a clinical and therapeutic point of view.

A little 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 will bind to the host cell's main receptor (ACE2). To do this, the spike must have 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, which allows the cleavage of spike between its S1 and S2 subunits. Spike is synthesized as an inactive precursor that must be cleaved for 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)

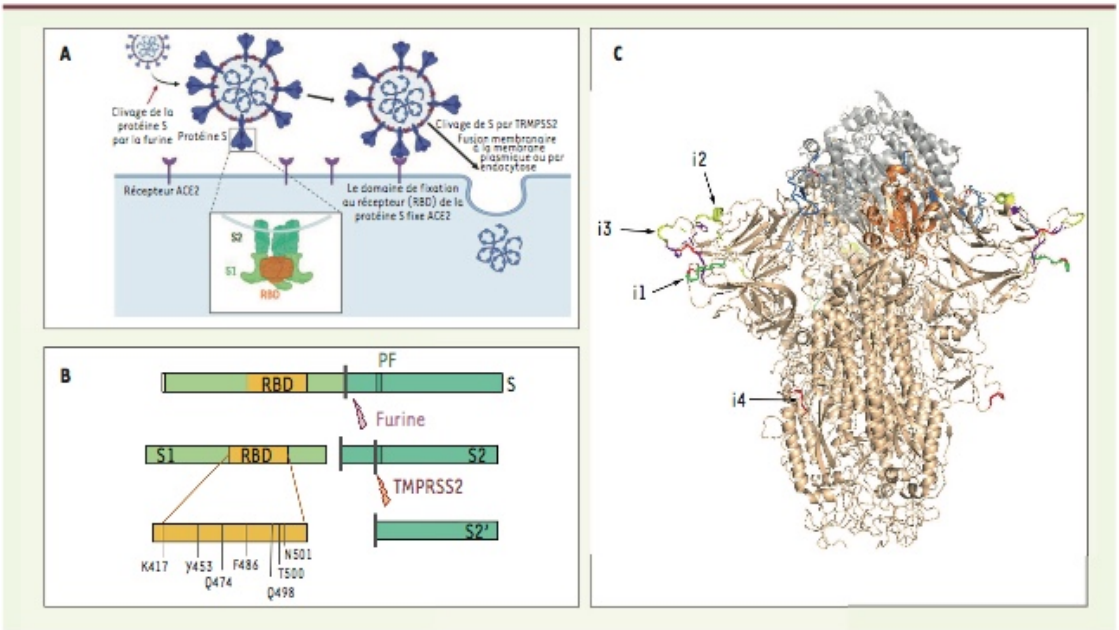


Figure 3. Structure et fonctions de la protéine S (spicule, spike en anglais). A. Représentation schématique de l'infection des cellules par le SARS-CoV-2 après fixation de la protéine S au récepteur ACE2. ***B.*** La protéine S subit deux étapes de maturation par clivage protéolytique (par les protéases 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 from "Tracing the origins of SARS-CoV-2 in coronavirus phylogenies".
Erwan Sallard, José Halloy, Didier Casane, Jacques van Helden, Etienne Decroly
https://www.medecinesciences.org/fr/articles/medsci/pdf/2020/07/msc200195.pdf

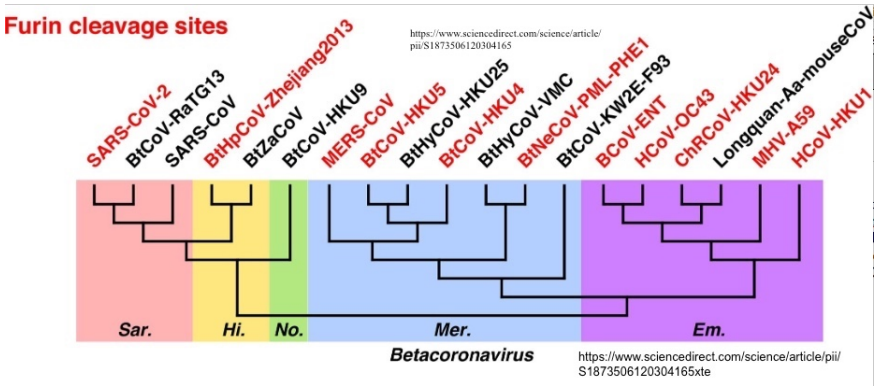
If we compare SARS-CoV-2 with its predecessors (the 2 coronaviruses that caused more localized epidemics, MERS and SARS-CoV of 2003), it is the only one to possess both this furin site and an RBD that binds to human ACE2 (MERS binds to another receptor (19) .

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]

Cellular receptors for different human pathogenic coronaviruses (Figure 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 site occurrence in human pathogenic coronaviruses (Figure from Wu Y, Zhao S. 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 2 modes of entry. It has been shown for both MERS-CoV spike and SARS-CoV-2 that the furin site at the S1/S2 junction promotes entry into lung cells, and contributes to viral pathogenesis in animal models of SARS-CoV-2 (20).

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

The affinity of the spike binding domain of SARS-CoV-2 for human ACE2 is higher than that of SARS-CoV (21). Early SARS-CoV-2 isolates were surprisingly well adapted to human ACE2, which may explain its rapid transmission. Human ACE2 has the strongest binding interaction, significantly higher than any of the species proposed as the source of the virus (22) . Rapid adaptation of SARS-CoV occurred during the SARS epidemic in 2002 and 2003: when SARS-CoV was transmitted from civets to humans, the spike gene underwent positive selection, in which mutations in two critical residues (amino acids 479 and 487) of the spike protein changed the binding affinity of the virus to human ACE2 from low to high, transforming it into a pandemic strain, following dissemination of the virus by a superspreader in which it could have acquired an increased transmission capacity (23) . The 2002 SARS-CoV-1 epidemic may have resulted from several different introductions from the wild (24).

Receptors other than ACE2

There are probably other cellular receptors than ACE2 that allow the infection of cells by SARS-CoV-2 (25): the protein LFA-1 (leukocyte function-associated molecule) expressed exclusively in leukocytes would allow the virus to enter 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 CD4 lymphocytes (26) . An article proposed it as early as April 2020, it was withdrawn by the authors under scientific pressure (27): it was not good to evoke a functionality close to HIV for SARS-CoV-2!
SARS-CoV-2 (like SARS-CoV, HIV-1 and Ebola virus) also uses DC-SIGN (dentritic cell-specific ICAM-grabbing non integrin) receptors to enter cells through spike glycans (28). Human respiratory cells in culture are also infected by binding to DC-SIGN.
DC-SIGN receptors are expressed on dendritic cell-derived monocytes and macrophages, LAF-1 on leukocytes (including T cells and DCs). DC-SIGNs are able to bind to the HIV-1 envelope glycoprotein gp120 and increase T cell infection. These 2 types of receptors are different but the overall architecture of their binding domains may be similar (29) .

Sequences of homologies with HIV

Montagnier-Perez hypothesis

They discovered (30) in SARS-CoV-2 a 225 nucleotide sequence which is absent in all coronaviruses (except RaTG13 which is certainly a laboratory construction). This sequence contains four HIV-specific regions (HIV1 and HIV2). For HIV1, the sequences are those of a strain that was used to create a candidate HIV vaccine.

The HIV Gp120 sequences bind to DC-SIGN (31) and the 3 are found on the spike-in binding sites of SARS-CoV-2. The 4th sequence has homology to the HIV gag protein and involves the furin site.

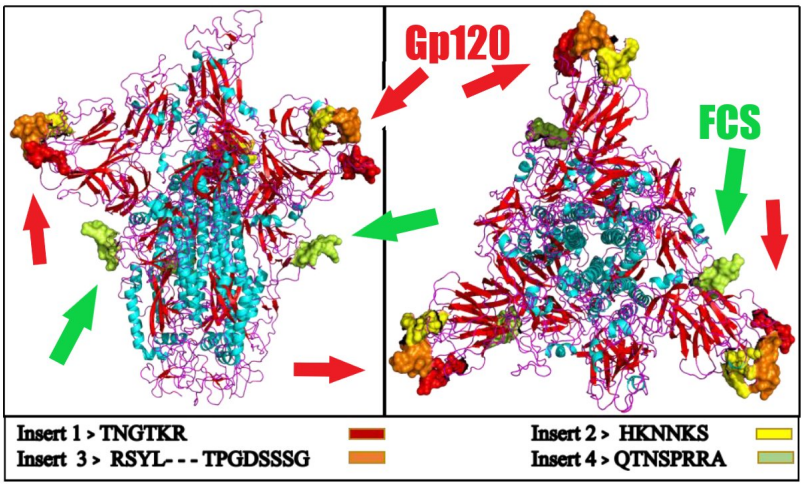


Figure 3. Modelled homo-trimer spike glycoprotein of 2019-nCoV virus. The inserts from HIV envelop protein are shown with colored beads, present at the binding site of the protein.

Figure taken from the retracted article by *et al*. (32): the HIV homology sequences are siutated on spike binding sites.

Prior to the first Montaginer-Perez publication, an Indian paper (January 31, 2020) withdrawn from publication due to criticism also showed the existence of these HIV homology sequences on SARS-CoV-2 spike binding sites (32). The authors withdrew the article on their own in the face of the attacks; they then checked their work and tried to republish but were refused by all the journals they contacted; however, their work was subsequently confirmed (33).

In summary, the Covid-19 virus has several molecular features 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 the HIV homology sequences (the HIV gp120 sequence binding to DC-SIGN is able to reactivate a latent HIV provirus. Since these homologous sequences are found in SARS-CoV-2 they could be responsible for reactivation of AIDS in infected individuals (34).

The different hypotheses on the origin

An article by 3 internationally renowned virologists (published in April 2021 in Virologie, 35) reviews the arguments in favor of a non-natural origin. The research programs conducted at the Wuhan Institute of Virology (WIV) by EHA and co-funded by the NIH are fully consistent 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 food or the cold chain, and finally a laboratory accident.

"These working hypotheses are classified from highly probable to very unlikely by the commission without any rational basis for this classification and while laboratory virus escapes have been documented in the literature.

The authors recall that viruses can only pass from bats to humans after a succession 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 bat to human is possible but extremely rare and does not cause an epidemic (it occurs by "spillover", when an individual is infected by very large quantities of virus as in the case of the miners of the Moijang mine (36).
No animal having played the role of intermediate host could be identified, this invalidates the strong 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
Laboratory virus escapes have been documented in the literature. For SARS-CoV, there are 3 documented cases of escape of this virus from P3 and P4 safety laboratories (Singapore, Taiwan and China, 37) . This also concerns other infectious agents (38) .
The H1N1 virus has escaped twice from a laboratory: in 1977 and in 2009 (it is at the origin of the 2009/2010 pandemic, probably due to a poorly inactivated vaccine, according to Furmanski (39).
As we shall see, the gain-of-function research programs conducted at the WIV and co-financed by the NIH are compatible with the artificial origin of the virus. Indeed, these gains of function concern the acquisition of the ability of the virus to bind to human ACE2 in order to anticipate the emergence of a potentially pandemic virus and to develop in advance the vaccine strategies to respond to it. Numerous experiments to insert basic amino acid-rich sites at the S1/S2 junction have already been performed to potentiate infection by CoV ("furin sites") (35 and see below for these experiments).
Unusual characteristics of SARS-CoV-2 suggest that it was manufactured in the laboratory and then adapted for human use in humanized mice, i.e. mice genetically transformed to express human ACE2, its cellular receptor (40).

Association of ancestral SARS-CoV-2 sequences with sequences from laboratory-grown cells: host genomes of the unique SARS-CoV-2 variant seeped into Antarctic soil metagenomic sequencing data
Researchers 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 contaminated the sequencing apparatus in Shanghai (41) . Sequences of SARS-CoV-2 isolated from the first Chinese patients were deleted from the databases and found on the internet by Jesse Bloom (42).
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 relative to Wuhan-Hu-1 is one of two plausible progenitors of all currently known human SARSCoV2. In the same sample are sequences that may have originated from the hosts that harbored these primitive SARS-CoV-2: human mitochondria, CHO (Chinese hamster ovaries) cells and Vero. The fact that these primitive sequences are close to those of RaTG13, that they were found associated with sequences from laboratory cells that may have been used to grow SARS-CoV-2, supports the hypothesis of a virus that escaped from a laboratory.

Arguments against natural origin
For coronavirologists (43) it is not possible to decide between natural and synthetic origin.
Bioinformatic analysis of the SARS-CoV-2 genome shows a bias in codon usage suggesting possible genetic manipulation: if the virus had appeared naturally, the proportion of each

codon in the genome would have been different because in natural viral RNAs the distribution of codons remains in fairly stable relative proportions (note 18).
This is confirmed by Ralph Baric (professor at the UNC -University of North Carolina and participant in the PREDICT and DEFUSE projects), who states that in 2019 it was possible to create a chimeric virus without leaving any traces, contrary to 2015 (44). Ralph Baric, author of the 2015 chimeric virus (45) stated:
- "You can create a virus without leaving a trace. The answers you seek, however, can only be found in the Wuhan laboratory archives."
- "In the chimera we made in America in 2015 with the SARS virus, with Professor Zheng-li Shi of 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 laboratory-made virus."
The sequences of the viruses' genomes can be downloaded, but the Wuhan databases are gone. Since 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 originated from a bat virus RaTG13, an isolate of which was reportedly collected in 2013 and stored at the Wuhan laboratory. RaTG13 would have been sequenced only in 2017-2018 and published in February 2020. This hypothesis seems completely made up in hindsight as the name of this strain was changed without explanation (BtCoV/4991 originally), and the RBD of RaTG13 has only 70% homology with that of SARS-CoV-2. There is a doubt about the veracity of the RaTG13 sequence because if it had really been collected from bat feces one would expect to find bat and bacterial RNAs in it, which is not the case.
Moreover RaTG13 has a receptor binding domain (RBD) capable of binding to human ACE2 (like SARS-CoV-1) which should have jumped out at Zengh-li Shi if she had really isolated it in 2013 and sequenced it in 2017-18: she would not have waited for the beginning of the SARS-CoV-2 pandemic to publish the RaTG13 sequence. The ratio of synonymous and nonsynonymous mutations and their distribution, as well as the sequence of the SARS-CoV-2 E protein, further signal an artificial synthesis of this genome (46) .
The natural origin is based on the spillover hypothesis (contamination of another species than the one to which a virus is adapted by a massive inoculum effect, this happened in a mine in China 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 the bat.
The pangolin hypothesis raises many questions: recombination would have occurred between a pangolin virus and a bat virus, however, no intermediate virus, which would result from this recombination could be identified to date.

How did we come to suspect more than strongly the artificial origin?

January 2020:
A Chinese bioinformatics team publishes in Chinese in January 2020: this is the first mention of the furin site (47). On January 31, 2020, an article withdrawn since and quoted above also alluded to it (32)
Researchers from two teams from Aix Marseille University, CNRS and Université de Montréal 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 multi-basic motif at the cleavage site of the HA hemagglutinin of the H5N1 virus was probably associated with the hypervirulence of the virus during the Hong Kong epidemic in 1997 (48) .

The common cold coronaviruses HCoV-OC43 and MERS possess the furin cleavage site in the spike. SARS-CoV-2 has an additional 12 nucleotides encoding a polybasic PRRAR site (amino acids 680-684 of the spike: ProlineArginineArginineAlanineArginine) corresponding to a furin cleavage site that represents a gain of function for this virus compared to other beta-coronaviruses, allowing it to spread in the human population. This was confirmed on 18 February 2020 by an American-French team (49).

February 2020:
Zheng-Li Shi of WIV publishes the alignment of amino acid sequences of the S1 subunit of the SARS-CoV-2 spike with other coronaviruses in Nature (50). 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 elsewhere than at the furin site. This allows him to conclude that SARS-CoV-2 originates 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 (Zheng-Li Shi would not have seen there the RBD able to bind to human ACE2, and did not publish it).
These two "omissions" are equivalent to a confession from Ms. Shi: we cannot expect her to publicly acknowledge having inserted this furin site! I don't like scientific metaphors, but it is 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 in a virus is infinitesimal or even null: 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 farm animal viruses that go back and forth between animals and humans). This argument is also given by Segreto et al. 2021(51) . The appearance of the furin site is not accompanied by other point mutations in the sequence (compared to previous natural viruses), which would be expected in natural evolution.

Furin site insertion experiments and ACE2-binding RBDs 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 part of the DEFUSE project: they concern the furin site, binding to ACE2 of 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 pseudovirus vaccine candidate to enter all mammalian cells, then these viruses would be self-destructed inside the cells (52) .
2008 - Ralph Baric constructs a virus chimera with an altered 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 spike and regions of S2. It was recalled that polioviruses and the influenza virus of 1918 had already been reconstructed with these techniques as well as retroviruses. Work financed by the 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 strongly increases the infectivity of coronaviruses (54).
Peter Daszak, co-author with Zheng-Li Shi of a 2013 paper (55) , justifies these experiments to prepare for future pandemics.

2014 - Ms. Zheng-Li Shi, director of the Center for Emerging Infectious Diseases at WIV, received more than $1.2 million from the US government between 2014 and 2019 (56) . She and Ralph Baric (57) created a chimeric virus by inserting the spike protein gene from a wild bat virus into the genome of an SARS virus that was adapted to multiply in mice and mimic the human disease.
2015 : Paper published in Nature Medicine (58) describing the creation of a chimeric virus by reverse genetics: a spike protein from 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 "backbone" adapted to mice. The chimeric virus can reproduce the SARS disease in mice and is able to infect human cells in culture. R Baric raises the question of the danger of this type of experiment: the risk of generating more dangerous pathogens is to be weighed against the potential to prevent future pandemics.
Scientists (one of them belonging to the Pasteur Institute) warn about the danger of these experiments.
A patent describing the modifications introduced into these chimeric viruses was taken in 2015 by Ralph Baric. It concerns a chimeric coronavirus spike protein modified for the RBD and the fusion domain; the furin site does not match that of SARS-CoV-2 (59) .
2017: the Shi-Daszak group published the creation of 8 chimeras from a virus collected from bats and different RBDs of SARSr (60)(SARS-like viruses) (EHA-WIV) . The need for furin for adaptation of coronaviruses to new species was known to R Baric since 2018 (61). 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-α (α-subunit of the epithelial sodium channel, a protein essential for airway surface fluid homeostasis, the misregulation of which is associated with respiratory disease (62)). This site has been studied at UNC (63) . It is optimized for arginine codons (CGG): these are the best codons found in humans for this amino acid.

Sequences of homologies with HIV

As said before, 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 spike binding sites of SARS-CoV-2. Only SARS-CoV-2 and RaTG13 (which is certainly a laboratory construct) contain these HIV sequences.
The furin site alone is not sufficient for SARS-CoV-2 pathogenesis: the upstream QTQTN amino acid sequence is required as well (64).
At the SARS-CoV-2 furin site (nucleotides 23548 to 23771, amino acids 677 to 686 - QTNSPRRARS), note that the QTNS sequence is derived from HIV and is adjacent to the PRRAR furin site sequence.
The corresponding HIV-1 Gag site is QTNS-silmqrsnfk-PRRA which happens to be identical to amino acids 366-384 of the gag protein cleavage site of an Indian HIV variant.

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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
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According to Sallard et al (40), HIV sequences may have arisen by natural evolution ("sequences sharing the same insertion appear clustered in the phylogenetic tree, suggesting a distinct origin for each insertion") and indeed the evolution of the peptide sequences can be followed here:
The Banal-52 strain was isolated in 2020 in Laos and does not contain the furin site (65).

These homolgies may have arisen by natural evolution and may have been optimized by Daszak and Baric (DEFUSE project).

Gain-of-function (GoF) debate (2011-2012)

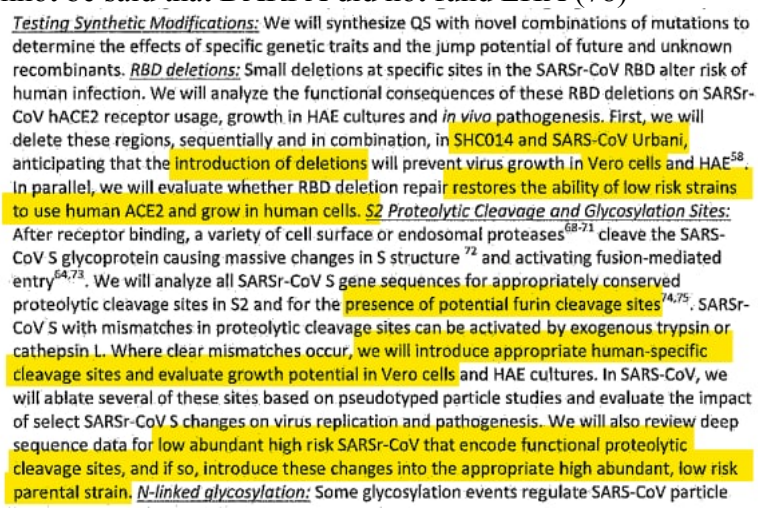
Gain-of-function (GoF) experiments consist in increasing the ability of an infectious agent to cause disease (through increased pathogenicity and/or transmissibility).
Following manipulations in animals on the H5N1 virus to anticipate its evolution allowing it to be transmitted from human to human, the attempts of American laboratories to publish their results launched a great debate on the so-called gain-of-function (GoF) experiments with pathogens having a pandemic potential (66) . A moratorium on GoF experiments with influenza, MERS, and SARS viruses was decided on October 17, 2014 in the US (67) : "No new US government funding will be provided for gain-of-function research projects 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 the testing would reasonably be expected to increase transmissibility and/or pathogenicity."
December 19, 2017: GoF Moratorium Lifted by NIH Director Francis Collins (68). The Secretary of the Department of Health, HHS (Health and Human Services) had resigned and the position remained vacant until January 2018; NIH and A Fauci took the opportunity to quietly restart GoF funding much to the astonishment of the scientific community.
Anthony Fauci has been Director of the NIAID National Institute of Allergy and Infectious Diseases since 1984, and has worked at NIH since 1968. He has defended gain-of-function research: he stated in 2012 that "the risk-benefit ratio of this research is clearly in favor of society" (69), for him moreover, "Nature itself is the most dangerous bioterrorist."

Senators Ron Johnson and Rand Paul were on this Committee on Homeland Security and Governmental Affairs; they returned to the fray in 2020 and 2021 against A Fauci over the NIH-funded coronavirus GOFs. One is struck by the similarity of the influenza virus and coronavirus experiments.
Peter Daszak, head of Eco Health Alliance, finances the GoFs (EHA, an NGO with an Orwellian profession of faith (70): "EcoHealth Alliance: Standing Between You and the Next Pandemic" the profession of faith has recently been modified: the previous one was ""Working for a world without pandemics"" (71). He was a member of the WHO Commission of Inquiry chaired by Peter Embarek, which investigated the Wuhan laboratory. This commission concluded that it was extremely unlikely that the virus had escaped from the laboratory. The extent of P Daszak's collusion of interests can be measured when one considers that he was a contractor, collaborator, and co-author of work conducted at 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 Wuhan P4 laboratory by one of its agents on site, there is mention of this document in the emails revealed by FOIA but it has not been published (72) .
In an interview published on the internet (73) P Daszak explains the experiments carried out by EHA "you can manipulate the virus in the laboratory, the spike protein is responsible for the ability of the virus to infect an animal, you can modify the sequence of the spike protein (build a protein), that's what we are doing with Ralph Baric, we are inserting the sequence of this protein in another virus. We are trying to develop a vaccine against this new virus that we are building to anticipate a pandemic.

According to Science (74), the NIH has funded $3.7 million in EHA for 5 years (some of the money was sent to WIV).
The EHA collaboration with WIV dates back to 2004, Peter Daszak has written 18 publications with Zheng-Li Shi.
Finally in a paper published in 2016 (75) , it is clear that GoF was conducted; 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 clear that NIAID spent this amount on GoF research to determine how bat coronaviruses can be made more pathogenic to humans, and that this research continued after the 2014 moratorium on such funding was implemented.

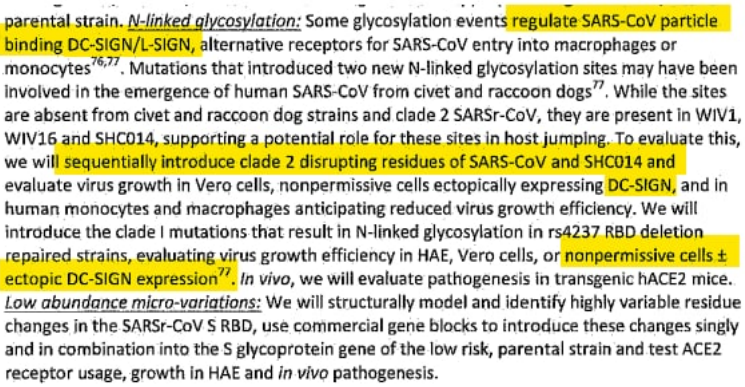
The latest declassified documents: aborted collaboration between EHA and the US Army : DARPA-DEFUSE

To protect its soldiers sent on expeditions from the dangers of future emerging viruses, the US Army, through the Defense Advanced Research Projects Agency (DARPA) issued a request for proposals (76) in 2018 for research to anticipate the emergence of future coronaviruses with pandemic potential. This call for proposals 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 to prevent the diffusion of these viruses from their animal reservoir that are bats (by suppressing this virus).
EHA responded to this call for proposals but its DEFUSE project (77) 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 spike binding domain (RBD) capable of binding to the human ACE2 receptor and a furin cleavage site (human ubiquitous enzyme).
However, DARPA pointed out that some of the proposed experiments could be funded, so it cannot be said that DARPA did not fund EHA (78)



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

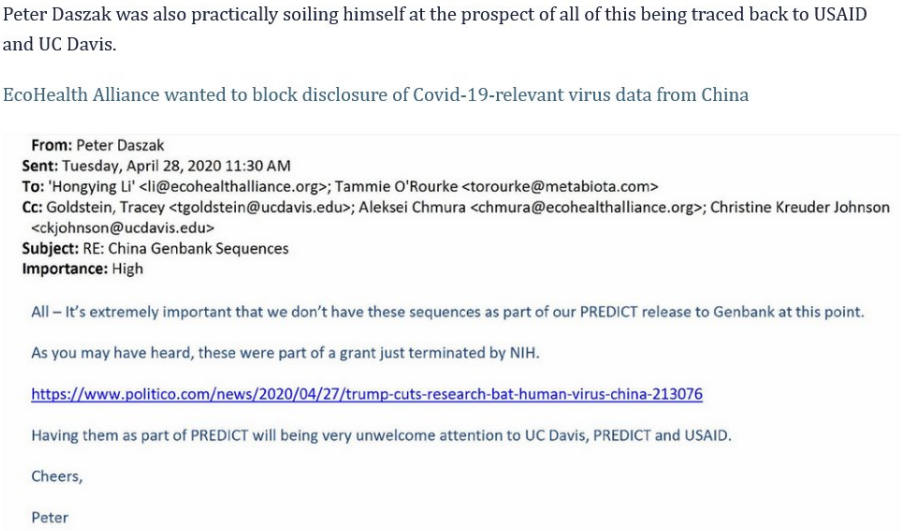
EHA proposed to synthesize spike proteins that bind to the human ACE2 receptor and to insert them into backbones (genomes) of SARSr-CoV (bat virus close to SARS-CoV), it was also question of inserting a furin cleavage site into the synthetic viruses (SARS-CoV do not have this site).



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.

On page 11, EHA emphasized the importance of SARS-CoV-1 spike glycosylation sites in binding to DC-SIGN receptors for entry into macrophages and monocytes; EHA proposed

to introduce into chimeric viruses the glycosylation-promoting mutations found in recently collected bat strains (WIV1, WIV16 and SHC014). These chimeric viruses would be introduced into cultured cells (in particular HAE, human airway epithelial cells) and their pathogenesis in mice would be monitored.



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 an April 2020 declassified email from P Daszak it states, "it is extremely important that we do not give away these sequences as part of the PREDICT publication in Genbank at this point." These are the sequences underlying the GP120 peptide sequences of SARS-CoV-2. That's why Pradhan only found the peptide sequences they were based on. The only nucleotide sequences found in GenBank or BLAST are those of HIV and SARS-CoV-2 and not those of the PREDICT project.

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

This is where a break in the logic of the reasoning occurs. The authors of the DEFUSE project anticipate the realization of their model as if they had control of "The Time Machine"!

The designers of the EHA project are so sure that they have modeled the future pandemic viruses that they plan to vaccinate bats against these viruses that have not yet appeared with these living synthetic viruses! They therefore consider that these viruses will appear naturally in bats and that these animals will have to be immunized to prevent them from transmitting them to humans.

To verify the possibility of vaccinating bats against this future virus, EHA proposes to use a live aerosolized virus; why not have tested this possibility with a non-humanized virus or with an inactivated virus or a pseudovirus unable to replicate?

Not only does the EHA propose forbidden gain-of-function experiments, but it also envisages disseminating viruses with pandemic potential by aerosol: we understand DARPA's caution! However, according to the declassified documents thanks to the DRASTIC group (76), the DARPA did not exclude to finance certain parts of the project if other financing were found: the refusal concerned especially the amount requested.

This research on pandemic risk coronaviruses was not exclusive to the United States and China: in a 2018 thesis defended at the Institut Pasteur in Paris, we learn that a furin site was inserted into the spike of HCoV-229E (a common cold coronavirus) but using a pseudovirus with an MLV (Murine Leukemia Virus) skeleton that is incapable of replicating: it is thus

possible to model the appearance of a virus with pandemic potential without taking the risk of causing a pandemic! (79).

Collaboration between NIH/NIAID and EHA

The DEFUSE project discussed above involves a DARPA solicitation, but EHA has a long history of collaboration with NIH. Funding for gain-of-function projects on coronaviruses dates back to the early 2000s.
R Baric has worked since the 2000s on SARS vaccine research and on determinants of coronavirus pathogenesis. His CV (80) includes 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 resulted in a publication in 2003 (81) . The aim was already to manipulate the SARS-CoV genome in order to study its pathogenic effect and to develop live attenuated vaccine candidates.
The HHS (Health and Human Services) considers that GoF experiments can be allowed when developing and producing vaccines against a potentially pandemic pathogen (82) , this document allowed the lifting of the moratorium on GoF established in 2014.
In the DEFUSE project, it is stated that some of the experiments have already been performed. The details with which the planned experiments are described has led some specialists to assume that they have been partly carried out. This is confirmed by the reports quoted above and revealed by the FOIA (Freedom Of Information Act) of The Intercept.
In the current contract between EHA and NIH/NIAID (83) , it is stated that EHA and WIVI (Wuhan Institute of Virology) have built at least 3 chimeric viruses using NIH funds.
One of them (SHC014 WIVI) is able to generate a more than 10,000-fold higher viral load and greater pathogenicity in humanized mice (carrying human ACE2) compared to the parental strain.
According to the agreement signed with NIH, EHA should have stopped these GoF experiments immediately (it was expected if a 1-log increase was found, here we are at 3-log) and briefed NIH. This agreement is from 2016. NIH Deputy Director Lawrence Tabak (NIH Deputy Director) acknowledges that EHA did not notify NIH of this 2018-2019 experiment that resulted in a possibly human pathogenic virus.
This confirms that the previous assertions of F Collins (NIH Director), A. Fauci and L. Tabak are false: NIH did fund GOF experiments in Wuhan.
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 states in 2022 that it is possible that the virus came from a lab leak, he admits that he does not know what Ralph Baric actually did regarding the furin site; he also admits that he did not tell the NIH the result of the humanized mouse experiments that showed that they got much more pathogenic strains as a result of the GoFs, but then he says that the NIH knew about the humanized mouse experiments conducted in Wuhan. (84)
The funding also involved experiments with MERS viruses modified to infect human cells and humanized mice (natural MERS has the furin site but not the receptor for human ACE2, the lethality of this virus is 30%). NIH/NIAID grant R01 AI110964 involved the mutation of MERS viruses such as NeoCoV and PDF-2180 to infect human cells: replacement of the MERS RBD with the RBD of different HKU4-related strains; recovery of chimeric viruses, infection of human cells from different tissues (lung, liver, intestine, kidney), replication in cells with the DPP4 receptor: results suggest a potential risk of infection of humans by these viruses. The close relatives of MERS-CoV in bats use ACE2 as a functional receptor (85) .

Further results have not been published: did the mutant viruses prove to be too dangerous, or, after infecting human cells, did the researchers give up on infecting humanized mice with a potentially pandemic virus once the emergence of SARS-CoV-2 was known? Experiments were also conducted with a swine virus, SADS-CoV, which is responsible for fatal diarrhea in China and 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 involved pseudoviruses and not replicative viruses (86): they are VSV-dG (VSV-based rhabdoviral pseudotyping system).
What would have happened if SARS-CoV-2 had not emerged and if GoF with MERS2 had been conducted on humanized mice or with bat vaccines?
This contract is still ongoing: in November 2018, EHA applied for a grant extension by NIH which was 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 was granted in June 2021 ($1.5 million). This included characterizing the spike binding domains and experimenting in vivo with their influence on animal/human transmission. (87)

It can be assumed that by 2017 this research on chimeric viruses was well underway since on January 10, 2017, Anthony Fauci announced that there would be a surprise epidemic during Donald Trump's term. 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 across the spectrum to listen, learn, and discuss how the next presidential administration can contribute to pandemic preparedness, global health security, and national preparedness and resilience (88).

NIH Collaboration with Moderna

Moderna received $25 million in funding from DARPA in 2013 to develop mRNAs that can be rapidly deployed in the event of a new pathogen emergence (89) . In total, by 2015, Moderna had negotiated $450 million in funding (some of which came from DARPA) for its mRNA research relating in particular to mRNA vaccines for Ebola, RSV, and other (unspecified) viruses(90) .

In 2017, S Bancel, Moderna's CEO, decided to redirect the company's research towards vaccines, following safety problems with mRNA therapies for rare diseases (low doses were not very effective and high doses were too toxic). Bancel predicted that Moderna could consider having taken off within 5 years (so in 2022) (91) .

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 DRASTIC (92) .
In 2019, a technology transfer and benefit sharing agreement between NIAID and Moderna was amended (93) . This is a 153-page confidential document that describes amendments to a collaborative agreement between NIAID and Moderna, signed from 2015; many passages are stricken.
Previously, Moderna and NIAID had long been working on mRNA vaccines but not coronaviruses.
There was also talk of stabilized membrane proteins in a prefusion configuration, but the furin site does not appear until 2019 (June) as well as vaccines regarding MERS (April 2019).
Why was the above amendment signed in April 2019 to include MERS trials in the Moderna-NIAID agreement?

The Moderna patent (94)
Amabati *et al*. found 100% homology between the 12-nucleotide sequence of the furin site of SARS-CoV-2 and a sequence filed in a 2016 Moderna patent (95) .
However, this sequence was not 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 protein for the repair of DNA mismatches, MSH3. The optimization for human expression likely has applications in cancers with mismatch repair deficiencies.
Moderna was working on anti-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 to the reverse complement of a proprietary MSH3 mRNA sequence is highly unusual. Potential explanations for this correlation need to be further explored.

How to link all these gain-of-function experiments: the key man seems to be Ralph Baric whose CV may shed some light (96)
R Baric received funding in 2005 to research live attenuated SARS-CoV vaccine candidates 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 which contains the HIV sequences.
SARS-CoV-2 could have been designed as an attenuated HIV vaccine or as described in Project DEFUSE as a model virus with pandemic potential or as an attenuated vaccine against all coronaviruses.
Other research on attenuated vaccines against HIV is in this direction.
Live attenuated vaccine (LAV) trials against HIV have been conducted in China with an attenuated influenza virus vector (97) .

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

SARS-CoV-2 has certain characteristics that could have been developed for a live attenuated vaccine: this type of virus must infect a host with lower pathogenicity and replication capacity than the wild-type strain it is designed to combat, while retaining immunogenicity.
The immune response is mediated by interferons, and SARS-CoV-2 has a particular sensitivity to α and β interferons (98). Several viral proteins involved in IFN signaling in SARS-CoV-2 appear to be affected by attenuation, such as NSP3, ORF3b and ORF6 (99) .

Codon optimization and deoptimization
Synonymous mutations have been used in the past as a strategy for virus attenuation through codon deoptimization (100).
Comparing RaTG13 with SARS-CoV-2, there is a clear accumulation of synonymous mutations in the spike around the furin site: the number of CpG dinucleotides in SARS-CoV-2 is significantly lower than in SARS-CoV or MERS and may also indicate attenuation. In contrast, the accumulation of CpG in the furin site region could direct an immune response to S in an otherwise attenuated virus (101).
Recombination resistance is a strategy for developing live vaccine candidates, as described by R Baric in 2018 (102). A LAV should also not mutate easily, and SARS2 appears to be quite resistant to mutation. This characteristic may have been achieved by selecting strains with an RNA-dependent RNA polymerase (RdRp) with improved fidelity (103).
Researchers with past experience in HIV have developed a candidate anti-Covid-19 vaccine based on the gag protein
They point out that the SARS-CoV-2 spike is very different from all other SARS: it carries an additional charge that strongly enhances its interaction with the DC-SIGN receptor, which

alone can mediate endocytosis (without the involvement of ACE2), which may explain the clinical evidence for its infectivity and pathogenicity (104).

This research on attenuated vaccines appears to be continuing:

Ralph Baric's team has synthesized and inoculated mice with mutant SARS-CoV-2 (105). These more pathogenic viruses for mice were obtained by serial passages in mice; they would have an attenuated profile in humans. These more pathogenic viruses were tested as a vaccine with a platform using pseudoviruses (virus replicon particle (VRP) (106).

The same teams (Menachery Galveston, Texas) that participated in the gain-of-function experiments continue to make modified viruses from SARS-CoV-2 and infect human cells, mice and hamsters with these viruses in order to make a vaccine with an attenuated virus (107).

The same was done by R Baric in 2020 (108). This team published the "recipe" for making SARS-CoV-2 by reverse genetics in order to study live attenuated vaccines, to facilitate serodiagnosis, vaccine evaluation and antiviral screening (109) .

Some researchers have clustered escape mutations on synthetic spikes embedded in pseudoviruses (110).

Such experiments could be the origin of the Omicron variant

There are various hypotheses, including the emergence in an immunocompromised patient with HIV in South Africa (111). The virus would have persisted for a long time until it acquired enough mutations to escape the immunity of the surrounding populations.

Murine and synthetic origins of Omicron

According to the team of F Balloux and L van Dorp (112), minimal adaptation was required for human-to-animal spread and subsequent transmission in mink and deer, underscoring the "generalist" nature of SARS-CoV-2 as a pathogen that readily infects a wide range of mammals.

The authors suggest that omicron may have arisen in mice (wild or laboratory?). The wild-type strain of SARS-CoV-2 cannot infect mice, but some variants can.

Omicron is not found in any intermediate human evolutionary branch suggesting that it evolved in an animal. Omicron has 5 mouse-adapted mutations. rare in clinical samples (Q493 and Q498 mutations are rare in clinical samples and Q498 is found in sewage host animals). Omicron must have evolved in an environment other than that of the previous variants: either in an immunocompromised patient or in an animal host.

The 5 mutations (K417, E484, Q493, Q498, and N501) increase the affinity for mACE2, they are found in the strain IA-501Y-MA-30 obtained after 30 IA-501Y passages in mice.

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 virus (MRCA) that was last seen ~April 2020, and accumulated more than 20 new spike protein mutations.

This means that 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 (113).

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 (114).

Results suggest that the Omicron progenitor moved from humans to mice, rapidly accumulated mutations favorable to infection of this host, and then returned 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 Omicron, a modification that appears to enhance furin processing during the viral life cycle (115).

It is possible to adapt SARS-CoV-2 to mice in 10 passages (R Baric) (116).

Towards SARS-CoV-3?

In November 2021, CDC, which is part of the Department of Health and Human Services (HHS), amended its agents and toxins regulations to add chimeric SARS-CoV/SARS-CoV-2 viruses resulting from any deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids encoding SARS-CoV virulence factors to the HHS list of selected agents and toxins.

This regulation is for biosafety laboratories classified as 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 chimeric virus with increased virulence. There is a significant potential risk of fusing a virus with known virulence factors with a pandemic virus, the resulting chimeric virus will have the transmissibility of SARS-CoV-2 and the pathogenicity of SARS-CoV (117) .

This is specific enough to suspect that this research is ongoing at the time of the regulatory change: it could lead to the emergence of the more pathogenic SARS-CoV-3!

Recent biopolitical developments

October 2019:

At a meeting regarding influenza vaccines, speakers (and in particular Anthony Fauci and Margaret Hamburg, foreign secretary of the National Academy of Medicine), half-heartedly proposed bypassing clinical trials of mRNA vaccines and, with the help of a disruptive crisis, bringing them to market without the need for ten years of testing (118) .

February 9, 2021

The WHO mission (119) to investigate the origin of the virus in Wuhan gives a press conference: the hypothesis of the escape of the virus from a laboratory is considered highly improbable given the absence of research projects involving coronaviruses close to SARS-CoV-2.

The mission chaired by Peter Embarek, Peter Daszak is part of it. February 14, 2021: Interview with Peter Ben Embarek, head of the WHO investigation mission in Wuhan on the origin of the virus.

Contrary to what was announced, this investigation has made it possible to no longer exclude an artificial origin of the virus, although this hypothesis is qualified as "very unlikely": it is said that before this mission this hypothesis was unthinkable.

It is also understood that the emergence of the virus well before December 2019 is not excluded: the Chinese collected 72,000 cases of influenza-like illnesses that appeared during 2019 and could have been due to Covid-19, but only 92 cases were retained for serological control and only 67 cases tested negative for SARS-CoV-2. It is not clear 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 (120)!

Letter from the US Congress, 11 January 2022

Two US Congressmen send a request to the Secretary of the Department of Health in Washington (121): the emails of Anthony Fauci 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 had failed to submit its 2019 report on NIH/NIAID-funded experiments: lawmakers speculate that this was to hide gain-of-function experiments on deadly and infectious new coronaviruses.
They suspect that Dr. Fauci had an article published in Nature Medicine about the origin of the virus changed in February 2020 (122): the authors of this article would have concluded that it was a laboratory leak.
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 genetic forcing projects and on self-dissiminating vaccines (Genetic forcing, Self-dissiminating vaccines, Chimeric viruses... Les apprentis sorciers du génome, Bruno Canard, Etienne Decroly & Jacques Van Helden, Le Monde Diplomatique, February 2022).

In the US, Senator Mike Braun will introduce an amendment on **April 4, 2022**, requesting HHS to release all documents related to WIV and the origin of Covid-19 (123).
During a March 2022 hearing, Senator Susan Collins stated that laboratory leakage is the most likely option; Senator Rand Paul referred to NIH-led function gains in Wuhan despite the moratorium that would have been circumvented (124) .
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 virus (125) .

Also in **March 2022**, 18 European scientists asked the Presidency of the European Commission to launch an investigation into the origins of Covid-19 similar to the independent Senate investigation in the US. They also ask that the documents related to the EU funding of the Wuhan laboratory (126) be published.
The Wuhan laboratory has been funded by the EU since 2015 (127) . A lack of communication was deplored by the European Commission, which had to temporarily stop payments in 2020 (128) . The EVAg project should, among other goals, anticipate the response to emerging viral diseases.

Conclusion

There is therefore a strong body of evidence that the virus is of artificial origin: it is thought to come from gain-of-function experiments conducted at 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 explanation of history.
And with this pandemic we have reached a higher stage: it is the interest of the whole system that is at stake, we are well beyond a plot.

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