THE TRUTH ABOUT GAIN-OF-FUNCTION RESEARCH

Granting new abilities to pathogenic microbes sounds dangerous, but what has the research told us? **By Amber Dance**

In Greek mythology, the Chimaera was a fire-breathing monster, a horrifying mishmash of lion, goat and snake that laid waste to the countryside. In 2015, virologists led by Ralph Baric at the University of North Carolina in Chapel Hill reported the creation of their own chimaera. They took a version of the coronavirus responsible for the deadly outbreak of severe acute respiratory syndrome (SARS) in the early 2000s — now known as SARS-CoV — and adorned it with surface proteins from a different coronavirus taken from Chinese horseshoe bats. In the laboratory, this particular mash-up was able to break into human cells and also make mice ill[1]. This chimaera came with a message: other coronaviruses have the potential to spark a human pandemic. In just a few years' time, that warning would prove prescient, as a distant cousin of SARS-CoV has now killed more than 4.9 million people worldwide.

"It probably didn't get the recognition it should have had from the general virology community and people involved in pandemic preparedness," says Katherine Spindler, a virologist at the University of Michigan Medical School in Ann Arbor, who was not involved in the work. "Hindsight is 20:20."

But the 2015 study did raise broad interest for another reason: some wondered whether such an experiment should ever have been attempted. The work was considered by some an example of 'gain-of-function' virology, in which scientists bestow new abilities on pathogens to study them.

The term first gained a wide public audience in 2012, after two groups revealed that they had tweaked an avian influenza virus, using genetic engineering and directed evolution, until it could be transmitted between ferrets[2,3]. Many people were concerned that publishing the work would be tantamount to providing a recipe for a devastating pandemic, and in the years that followed, research funders, politicians and scientists debated whether such work required stricter oversight, lest someone accidentally or intentionally release a lab-created plague. Researchers around the world voluntarily paused some work, but the issue became particularly politicized in the United States.

US funding agencies, which also support research abroad, later imposed a moratorium on gain-of-function research with pathogens while they worked out new protocols to assess the risks and benefits. But many of the regulatory discussions have taken place out of the public eye.

Now, gain-of-function research is once again centre stage, thanks to SARS-CoV-2 and a divisive debate about where it came from. Most virologists say that the coronavirus probably emerged from repeated contact between humans and animals, potentially in connection with wet markets in Wuhan, China, where the virus was first reported. But a group of scientists and politicians argues that a laboratory origin has not been ruled out. They are demanding investigation of the Wuhan Institute of Virology, where related bat coronaviruses have been extensively studied, to determine whether SARS-CoV-2 could have accidentally leaked from the lab or crossed into humans during collection or storage of samples.

The arguments have highlighted questions about gain-of-function (GOF) research. But the classification is hard to define precisely. "What we mean by the term depends on who's using the term," says Gerald Keusch, associate director of the National Emerging Infectious Diseases Laboratories at Boston University in Massachusetts.

Here, *Nature* attempts to elucidate what constitutes GOF, and what science and medicine can learn from it.

The meaning of GOF

What is GOF? Debate over that question got heated at a US Senate hearing in July, when Senator Rand Paul (Republican, Kentucky) and Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases (NIAID), went head-to-head over a 2017 paper[4] by scientists at the Wuhan Institute. NIAID had supported the research through a New-York-based organization called EcoHealth Alliance. And it had done so at a time when funding for some GOF science was barred. The authors genetically grafted spike proteins — the viral keys that grant access to mammalian cells — from eight different, naturally occurring coronaviruses onto another coronavirus from the wild, called WIV1. They found that these new creations, in lab dishes, could infect monkey kidney cells, as well as human cells, through the same gateway — the widely expressed ACE2 receptor — that is used by SARS-CoV and SARS-CoV-2.

Senator Paul insisted that the work constituted GOF. Fauci was adamant that it did not.

It's no surprise that politicians and scientists would disagree on GOF's meaning, because it can mean different things in different contexts.

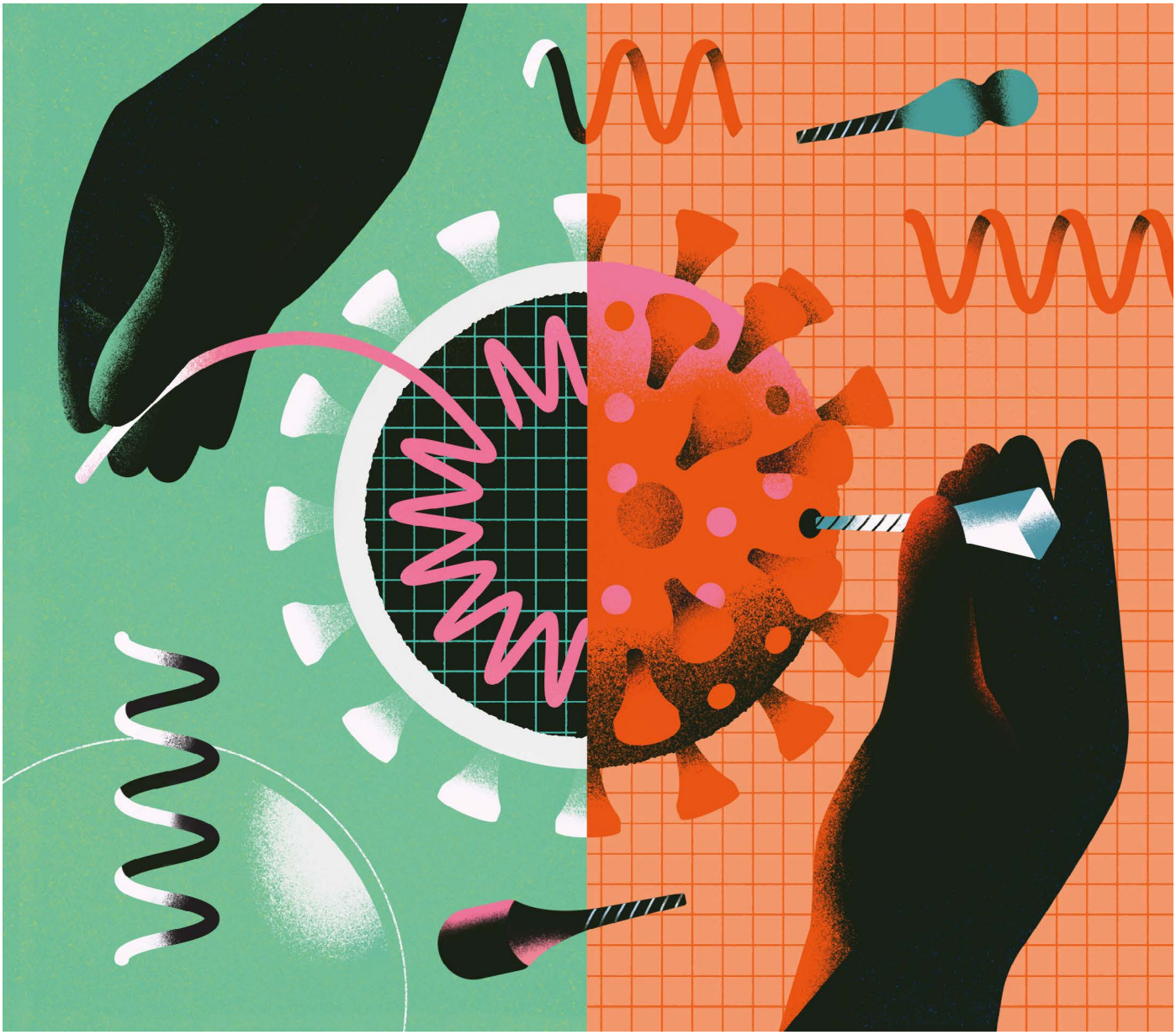


ILLUSTRATION BY KASIA BOJANOWSKA

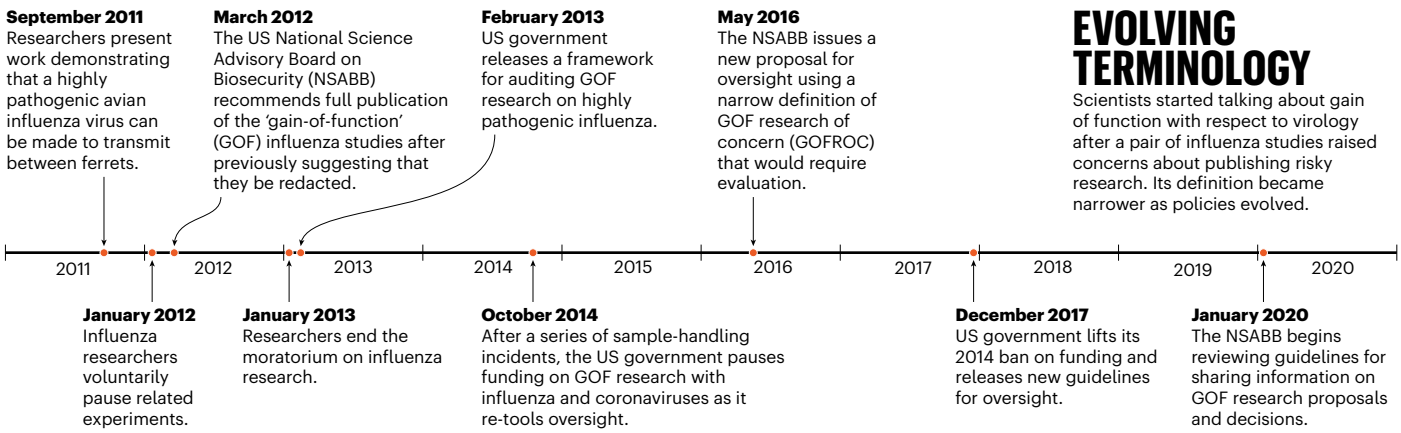
"WHAT WE MEAN BY THE TERM DEPENDS ON WHO'S USING THE TERM."

At its most innocuous, GOF is a classic genetics term to describe mutations that give a gene, RNA or protein new abilities or expression patterns. Gain of function might result in bacteria that are extra sensitive to potassium ions[5], for example, or an *Arabidopsis* plant with short stems and curly leaves[6]. A complementary approach – loss-of-function – involves disabling a gene to see what happens to organisms that lack it.

The term GOF didn't have much to do with virology until the past decade. Then, the ferret influenza studies came along. In trying to advise the federal government on the nature of such research, the US National Science Advisory Board for Biosecurity (NSABB) borrowed the term – and it stuck, says Gigi Gronvall,a biosecurity specialist at the Bloomberg School of Public Health at Johns Hopkins University in Baltimore, Maryland. From that usage, it came to mean any research that improves a pathogen's abilities to cause disease or spread from host to host.

Virologists do regularly fiddle with viral genes to change them, sometimes enhancing virulence or transmissibility, although usually just in animal or cell-culture models. "People do all of these experiments all the time," says Juliet Morrison, a virologist at the University of California, Riverside. For example, her lab has made mouse viruses that are more harmful to mice than the originals. If only mice are at risk, should it be deemed GOF? And would it be worrying?

The answer is generally no. Morrison's experiments, and many others like them, pose little threat to humans. GOF research starts to ring alarm bells when it involves dangerous human pathogens, such as those on the US government's 'select agents' list, which includes Ebola virus and the bacteria responsible for anthrax and botulism. Other major concerns are 'pathogens of pandemic potential' (PPP) such as influenza viruses and coronaviruses.



"For the most part, we're worried about respiratory viruses because those are the ones that transmit the best," says Michael Imperiale, a virologist at the University of Michigan Medical School. GOF studies with those viruses are "a really tiny part" of virology, he adds.

But this little slice of the field became the focus when the NSABB talked about regulating or monitoring GOF research (see 'Evolving terminology'). After the ferret flu studies were eventually published, researchers and regulators struggled to determine what sorts of experiment should receive extra scrutiny as a potential biosecurity risk.

In 2016, the NSABB attempted to clarify matters with a new term, 'gain-of-function research of concern' (GOFROC). This category, the committee said, is research that would make a pathogen likely to spread widely or cause significant disease in humans. This, the committee decided, was the only type of GOF work so risky it would require extra regulatory oversight[7]. In 2017, the US Department of Health and Human Services (HHS) adopted this approach when it devised its framework for reviewing grants on pathogens with pandemic potential.

However, as the war of words between Paul and Fauci shows, the terminology is still hotly debated. The chimeric viruses in the Wuhan Institute study were new viruses made in the lab. But the manipulations that made them did not enhance their ability to cause disease in humans. The starting virus, WIV1, could already infect human cells using ACE2. Although some scientists have argued that the work does constitute GOF, at the time the research was approved, it was evaluated by NIAID and considered exempt from the funding pause.

Last week, leaders at the US National Institutes of Health (NIH) told the US Congress that EcoHealth Alliance had not informed the agency about experiments in Wuhan in 2018 that enhanced the virulence of WIV1 in mice, and that immediately reporting such findings was a condition of the funding. A representative for EcoHealth Alliance says that the data were reported in 2018 and that the organization is working to "promptly address what we believe to be a misconception about the grant's reporting requirements and what the data from our research showed". Both EcoHealth Alliance and the NIH have stated that the viruses in question had no role in the emergence of SARS-CoV-2 and that the research doesn't constitute GOF. But the continued controversy has set off more questions as to whether such research is warranted – and prompted more calls for transparency in how it is reviewed and approved.

Notably, the committee at the HHS charged with reviewing potential GOFROC work has not publicly released any of its deliberations (although details of grant review are typically kept private).

Only a handful of countries even have national policies on oversight for potentially risky biomedical research. And although China has long been a participant in international treaties and conventions on biosecurity, the nation didn't pass sweeping legislation until 2020. Its law, which took effect this April, requires approvals for research with highly pathogenic microbes by provincial departments of health or rural affairs. But the law does not specifically address GOF studies, and some experts say the rules are vague[8].

How GOF can help

Despite the ongoing debate, plenty of studies have imparted new functions to viruses, with clear benefit to science and medicine. Since the time when poliovirus was first grown in cultured cells, scientists have adapted viruses to live in culture. This enables production of a large supply of viral material for further study or for vaccine development. This process sometimes diminishes the pathogens' ability to make humans ill. After all, the lab dish contains no immune system, so viruses can streamline their life cycles by dumping costly activities that would normally protect them from host attack, says Stanley Perlman, a physician and virologist at the University of Iowa in Iowa City.

Scientists have also directly modified viruses to create vaccines; the COVID-19 shots from both Oxford–AstraZeneca and Johnson & Johnson are based on adenoviruses harmless to humans that were modified to produce the SARS-CoV-2 spike protein. Researchers have also altered viruses to deliver gene therapies or cancer treatments. "All that great stuff that is going to benefit humanity is a gain of function," says Vincent Racaniello, a virologist at Columbia University in New York City.

In addition, scientists routinely give viruses the ability to infect new hosts. Animal research – although fraught with its own set of ethical quandaries – allows scientists to study how pathogens work and to test potential treatments, a necessary precursor to trials in people. That's what Perlman and his collaborators had in mind when they set out to study the coronavirus responsible for Middle East Respiratory Syndrome (MERS-CoV), which emerged as a human pathogen in 2012. They wanted to use mice, but mice can't catch MERS.

The rodents lack the right version of the protein DPP4, which MERS-CoV uses to gain entry to cells. So, the team altered the mice, giving them a human-like version of the gene for DPP4. The virus could now infect the humanized mice, but there was another problem: even when infected, the mice didn't get very ill. "Having a model of mild disease isn't particularly helpful to understand why people get so sick," says collaborator Paul McCray, a paediatric pulmonologist also at the University of Iowa.

So, the group used a classic technique called 'passaging' to enhance virulence. The researchers infected a couple of mice, gave the virus two days to take hold, and then transferred some of the infected lung tissue into another pair of mice. They did this repeatedly – 30 times[9]. By the end of two months, the virus had evolved to replicate better in mouse cells. In so doing, it made the mice more ill; a high dose was deadly, says McCray. That's GOF of a sort because the virus became better at causing disease. But adapting a pathogen to one animal in this way often limits its ability to infect others, says Andrew Pekosz, a virologist at the Bloomberg School of Public Health.

The experiments did make the virus amenable to research, however, and the team shared both it and the engineered mice with others. It led to plenty of new findings. For example, Perlman's team discovered that an immune-system protein called interferon

fights the virus, at least in a very specific time window[10]. This parallels responses in people with SARS-CoV-2, suggesting that if interferon is provided as a treatment, it should be early in the course of the disease[11].

Researchers also used the mouse-adapted MERS-CoV to test new vaccines and treatments. The Iowa team's collaborators tested a vaccine that is a hybrid of parainfluenza virus with the MERS-CoV version of the spike. The vaccine wasn't very effective when injected, but it did protect DPP4-expressing mice from MERS-CoV quite well when provided through the nose[12]. Although MERS outbreaks haven't led to sustained transmission, this information has proved valuable in the COVID-19 pandemic: a vaccine with the same design, but against the SARS-CoV-2 spike, works in mice and ferrets[13] and is now undergoing early clinical trials.

The big questions about GOF

Treatments and vaccines are clear benefits, but what has the body of scientific knowledge gained from basic-science experiments that skate close to, if not cross into, GOF or GOFROC territory?

The initial set of experiments that made GOF a household name revolved around avian influenza, a type known as H5N1. People sometimes catch it from poultry, and it can be fatal, but humans don't typically transmit the virus to one another. Scientists wanted to know, however, what it would take to make that happen. "That's the kind of question you can only answer with a gain-of-function experiment," says Angela Rasmussen, a virologist at the University of Saskatchewan Vaccine and Infectious Disease Organization in Saskatoon, Canada.

Teams in the United States and Japan[2] and the Netherlands[3] set out to test whether the bird virus could possibly evolve into something that's transmissible between mammals, in this case ferrets, which are vulnerable to infection by respiratory viruses and are a common animal model in flu studies. The researchers' strategies included making both specific and random mutations in the viral genome as well as passaging the pathogen through a series of ferrets to encourage it to adapt to the new host — much as McCray and Perlman did with MERS-CoV in mice. The result was that, yes, there are genetic changes that can make avian flu spread from ferret to ferret[2,3]. The new viruses were weakened and non-lethal, but they sparked a considerable fuss as science funders, regulators, journals and others debated and deliberated whether the data should even be published.

> "HAVING A MODEL OF MILD DISEASE ISN'T PARTICULARLY HELPFUL TO UNDERSTAND WHY PEOPLE GET SO SICK."

As for whether it was worth it, opinions differ. "Their practical importance, wasn't, in my mind, very extraordinary," says David Morens, senior adviser to the director at NIAID. "They don't help us answer the questions of whether H5N1 might become pandemic or what we would need to do to recognize or prevent it." (The corresponding authors of these two studies did not respond to, or declined, interview requests from *Nature*.)

Imperiale thinks otherwise: "We learned the determinants of mammalian transmission," he says. For example, the work supported suspicions that for a flu virus to infect a mammalian host, it must adapt to the temperature of the host's lungs and to the pH of that host cell's interior compartments.

The studies also identified several specific mutations that might allow an avian flu virus to turn into a mammalian flu virus, something scientists could watch out for in bird populations. The value of that is somewhat speculative, however. Surveillance of farmed and wild birds is far from universal and avian influenza could, theoretically, evolve to infect humans by an entirely different set of mutations.

As for coronavirus, Baric's 2015 chimaera experiments used a version of SARS-CoV adapted to infect mice, not people, so it might not fit the strictest definition of GOFROC. The chimaera, with the spike from a wild coronavirus called SHC014, was no better at infecting human cells than the original mouse-adapted SARS[1]. Baric did not respond to interview requests from *Nature*, but at the time, the authors wrote that "scientific review panels may deem similar studies building chimeric viruses based on circulating strains too risky to pursue". More recently, Baric told *MIT Technology Review* that the work should not be considered gain of function. "We retained function," Baric said — or even lost function, in that the virus was less able to make mice sick than the original SARS virus.

Baric's studies, and the similar ones carried out by researchers in Wuhan that Rand Paul was concerned about[5], did predict that a coronavirus could jump to humans and cause a pandemic, years before they were proved right. But the benefits go beyond that, says Rasmussen. "The irony is, these experiments and the work that was done at the Wuhan Institute of Virology, I think, really gave us a lot more information about SARS-CoV-2 than we would have had."

For example, Baric has pointed to work in his lab suggesting that remdesivir, a drug then in development to fight Ebola, would be a potential treatment for coronavirus infection. In this case, no chimaeras were involved, but the researchers worked with a handful of naturally occurring bat coronaviruses they'd reconstructed in the lab. The team found[14] that the drug protected human cells from these viruses as well as from SARS-CoV and MERS-CoV. It also reduced symptoms in a mouse model infected with SARS-CoV. Remdesivir was quickly applied to people with COVID-19 and is so far the main antiviral in use — although clinical results have been mixed.

Globally, GOFROC experiments will probably continue to be a rarity. But some virologists can envisage valuable experiments with SARS-CoV-2 that could be considered GOF. For example, Morrison thinks that experiments to look for mutations that make SARS-CoV-2 resist vaccines or treatments could be beneficial, so that scientists can be better prepared if such variants emerge. And as scientists attempt to make vaccines that work on all coronaviruses, it might be useful to test the vaccines' abilities to protect against infection by chimeric viruses that incorporate spikes from various wild specimens, suggests Stephen Goldstein, who studies viral evolution at the University of Utah in Salt Lake City. In fact, Baric has already tested one potential vaccine against a hybrid of mouse-adapted SARS-CoV and a bat coronavirus[15].

The ongoing political debate has meant that some virologists dare not even propose research that might be deemed GOF, says Pekosz. Some are even afraid to talk about it publicly. A survey by *Nature* published earlier this month suggests that scientists who speak out on topics related to the origins of COVID-19 are subjected to high rates of harassment.

With all the challenges inherent in GOF studies, why do them? Because, some virologists say, the viruses are constantly mutating on their own, effectively doing GOF experiments at a rate that scientists could never match. "We can either wait for something to arise, and then fight it, or we can anticipate that certain things will arise, and instead we can preemptively build our arsenals," says Morrison. "That's where gain-of-function research can come in handy."

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