Review Article

A Comparative Analysis of Gain-of-Function Research and Future Perspectives

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ABSTRACT

Gain-of-function (GOF) research is both impactful and controversial, as it involves genetically altering a pathogen to enhance its biological functions. One side believes that GOF research can offer knowledge about deadly pathogens and allow scientists to prevent future outbreaks. However, the other side argues that GOF research risks causing pandemics, making it too dangerous. There is clear disagreement in the scientific community regarding GOF research. This paper presents a comprehensive analysis of the GOF research debate by comparing the arguments and presenting the common grounds between them, as well as the limitations of current literature. Both sides prioritize protecting humankind yet emphasize the need for public involvement in the GOF research debate. Due to the pressing and significant need to make a decision regarding the future of GOF research, more objective papers with updated arguments and data are needed for both sides of the debate, and more effort should be put forward to inform the public so they can be involved in the discussions.

Keywords: Gain-of-function research; COVID-19 gain-of-function; H5N1 gain-of-function; Mutation; Vaccine development

INTRODUCTION

Gain-of-function (GOF) research refers to experiments that aim to help scientific communities to have a better understanding of pathogens, their relationship with hosts, and how they cause diseases (1, 2). These goals are achieved by artificially adding a new function to the pathogen that will be unlikely or take a long time to emerge naturally without human interference. However, GOF research encompasses a broad range of research (2, 3). The more specific sector of GOF research raising great biosafety and biosecurity concerns is called GOF research of concern or enhanced potential pandemic pathogen (2). The results of GOF research can be pathogens with increasing transmissibility and virulence (1). GOF research can be used to study pathogens' pathogenesis and virulence and their interactions with hosts (3).

One of the classical examples of GOF research was the study of H5N1 influenza virus near the end of 20th century. Even though H5N1 influenza virus originated from birds, people were worried about the potential mammalian

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transmissibility, considering that a resulting pandemic could have a case fatality rate over 60% (4). Out of great concern, researchers went on to study the possibility of H5N1 virus to transmit among ferrets, which were thought to be the best animal models, by engineering the virus strains. By 2012, it was formally announced that the H5N1 virus could transmit between mammals through respiratory droplets. The study results caused panic and horror since scientists inadvertently assisted the highly pathogenic H5N1 virus to be more virulent among humans. In fact, the H5N1 influenza virus research was temporarily halted in 2012 due to the associated risks (5). This study brought people's attention to the potential issues of GOF research for the first time (4).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused COVID-19 pandemic in 2019 (6, 7). COVID-19 brought more than 7 million deaths across the world (8). Patients infected with COVID-19 showed symptoms like fever, chills, cough, and breathing difficulty (7). Severe cases could result in the use of ventilator support. To suppress the deadly infection of SARS-CoV-2 (the virus that causes COVID-19), many researchers and companies aimed to quickly produce effective vaccines against the disease (6).

Among the companies, the Johnson & Johnson developed a coronavirus vaccine by inserting spike protein genes into human adenovirus type 26 vector to generate immunity against SARS-CoV-2 (9). The replication gene in adenovirus is deleted to prevent it from replicating and causing infections in humans (7). Since the coronavirus spike protein gene was not in the adenovirus in the natural state, the act of artificially inserting the gene into adenovirus made the vaccine a typical example of the GOF approach to develop countermeasures against prevalent diseases (7, 9). As a lead vaccine candidate, Johnson & Johnson had its combined Phase I and II trials in July 2020 to speed up the vaccine development process (7). After preliminary Phase III results releasement, it was approved as an Emergency Use Authorization in U.S. and approved for use in many other countries like South Africa. Without the rapid vaccine development using the GOF approach, more people would be in danger of being infected with the deadly COVID-19 in the early stages of the pandemic.

In comparison, another potent vaccine developed in the early stage of COVID-19 pandemic Pfizer had a different mechanism of action (7, 10). The vaccine contained a nucleoside-modified mRNA, which encoded for SARS-CoV-2 spike glycoprotein and was delivered into host cells in lipid nanoparticles. Pfizer was considered an mRNA vaccine and not a gain-of-function vaccine approach since it did not involve the process of artificially modifying to increase a pathogen's virulence (7, 10).

Despite GOF research providing a crucial vaccine during the pandemic, the U.S. House of Representatives formally announced a ban on federal funding for GOF research that modified dangerous pathogens and could cause more harm to humans in 2023 due to concerns of leaking genetically modified pathogens into the public (11). This was an amendment on the previous policy and implied that NIH would stop funding GOF studies on potential pandemic pathogens all over the world, including the ones in the U.S. On the other side of the world, the European Union research funding calls were held after the COVID-19 pandemic. In the calls, issues like improving pandemic preparedness and response and better understanding host-pathogen interactions of infectious diseases with epidemic potential were addressed and granted funding (12). Instead of promulgating bans on potential pandemic pathogens research, the European Union seemed to encourage more studies on pathogens and causes of potential pandemic.

In the scientific community, a similar situation of opposing views on GOF research, especially after the COVID-19 pandemic, can be seen as well. There are constant debates going on regarding whether GOF research and experiments should be continued. There are scientists who strongly believe that GOF experiments are essential and irreplaceable in regard to learning the relationships between hosts and pathogens, pathogen infection mechanisms, and developing potential disease countermeasures. On the contrary, scientists who argue that GOF research should be halted emphasize that GOF experiments are less productive and costly and have great potential to cause deadly pandemics, so the risks of conducting such studies largely outweigh the benefits.

In spite of the heated debate going on in the community regarding the GOF experiment, navigating benefits and harms of conducting GOF experiments and both sides of the argument could be difficult with the quick advancement in life science field. In this paper, we will present the opposing arguments for the GOF experiments, identify the overlap area which both sides agree on, and analyze some limitations and assumptions made by the two sides. This will give a more complete presentation of the arguments for GOF experiments, especially to the non-scientists in the community. More accessible education will help non-scientists to have a better understanding of the problem and contribute to the community by making rational decisions and suggestions.

BENEFITS OF GOF RESEARCH

Correlations between Genotype and Phenotype of Pathogens

The power of GOF research stems from experimentation, where new knowledge can be gained through the direct study of biological systems, without artificial modification or involvement. One of the most significant pieces of knowledge that can be obtained from GOF research is correlations between genotype and phenotype of the pathogens (Fig. 1) (13). Since experiments are directly conducted on the real pathogens, any results and data observed about pathogens and their hosts can tell us what they will be like in real life. On the other hand, when doing similar experiments using computer models, scientists can still get somewhat reliable data (14). Considering that only a fraction of the complex biology is understood, and the modeling software is designed based on what people already know, scientists cannot guarantee that there will be no difference between the results predicted by modeling approaches and what is observed from experimentation with pathogens. The computational models still have many limitations and necessary assumptions made for simplicity, so they are no way near comprehensive or flawless and thus require experimental validation. In conclusion, through modifications to the genotype or physiological mechanisms of pathogens, scientists can demonstrate causal relationships between genes and biological traits that might otherwise be missed if only tested via modeling (13).

Pathogen Virulence and its Relationship with Hosts

Understanding the relationships between genes and biological traits helps enhance the understanding of pathogen virulence and its relationship with hosts (Fig. 1). Pathogenicity islands are great examples. Pathogenicity islands were discovered to be genetic elements on the chromosomes that are responsible for the bacterial pathogen virulence (15). They are only present in pathogenic bacteria strains, further implicating their significance in increasing pathogen's virulence. Scientists are trying to understand more about the specific gene functions in the bacterial pathogens and the disease pathogenesis. From that, they can develop potential disease treatments targeting certain regions in the pathogenicity islands. Up until 2022, 40-60% of predicted human gastrointestinal bacterial genes have unknown functions, so there is still a lot to discover (16).

Pathogenic Disease Transmission and Infection Mechanisms and Countermeasures

Furthermore, apart from being able to decode the functions of more genes, GOF experiments enable scientists to conduct pathogen adaptation experiments, where scientists track how pathogens evolve to become more fit for the environment over time. These experiments further deepen the understanding of disease transmission and infection mechanisms and bring more development of countermeasures and preventions (Fig. 1) (13). As discussed previously, the Johnson & Johnson coronavirus vaccine could be developed in the early stage of the

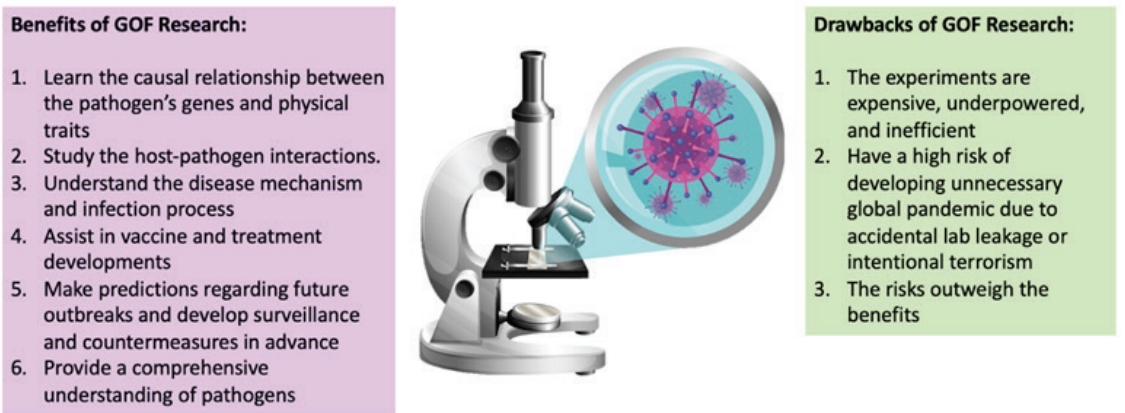

Figure 1. Benefits and Drawbacks of GOF Research (13). Each of the drawbacks and benefits was established from the literature explaining the benefits and cons. While not comprehensive, this generates a general view of the landscape of the GOF research.

COVID-19 pandemic because there had been decades of GOF research on adenovirus-based vaccines (1). Without the continuous GOF research before the pandemic, the development of a completely new vaccine with similar efficacy as the one by Johnson & Johnson would have undoubtedly taken several years. In summary, the enhanced understanding of pathogens and interactions with hosts gained through GOF research helps accelerate the development of effective vaccines and treatments.

Future Outbreaks Prediction

The ultimate goal of understanding pathogens is to protect the public from disease. One of the most important benefits of GOF experiments is being able to predict future outbreaks and design countermeasures in advance, minimizing case fatality rate of the later pandemic (Fig. 1) (3). In 2015, researchers engineered a chimeric virus that expressed the spike protein of bat coronavirus in a mouse-adapted SARS-CoV backbone (17). As severe acute respiratory syndrome coronavirus found in bats can cause deadly outbreaks, the study was conducted in a mouse model to examine the possibility of cross-species transmission of the virus. Since the original virus was engineered to be more pathogenic, the study was considered GOF research. They found out that the hybrid virus was able to replicate efficiently in primary human airway cells. At the time, all therapeutics had poor efficacy and protection against viral infections. Thus, the study result urged the community to develop efficient therapeutics and prepare for the potential SARS-CoV reemergence from the viruses circulating in bat populations at that time. In 2019, the COVID-19 pandemic took place (18, 19). The SARS-CoV-2 virus spread quickly in different countries and caused a severe pandemic. 232 countries have reported COVID-19 cases to World Health Organization, and more than 775 million infected cases have been reported worldwide (8). The SARS-CoV-2 could transmit from person to person through respiratory droplets which infected airway cells, which contributed to the quick spread of the virus (20). These COVID-19 details accorded with the future pandemic prediction made in the 2015 study, which showed that GOF experiments can indeed foretell future outbreaks.

It is worth noting that GOF experiments do not necessarily create new pathogens that cannot evolve in nature. It is argued by some scientists that GOF experiments mainly presage what will eventually happen in nature. They help scientists study the possible pandemic-causing pathogens and think of countermeasures in advance to the future outbreaks (21). Overall, GOF experiments provide a comprehensive understanding of pathogens, disease pathogenesis, and treatments that cannot be fully shown by other methods (13).

DRAWBACKS OF GOF RESEARCH

High Cost and Long Duration

The first drawback of GOF research is that it is expensive, rare, and low-throughput (Fig. 1) (13). Since GOF research mostly contains genetic editing of the pathogens to increase their pathogenicity or transmission, the materials needed for GOF research at least include gene editing tools (like CRISPR kits), genome sequencing, and other fundamental lab equipment (like PCR, gel electrophoresis, genome sequencing machine, etc.). Just for the DNA sequencing, it is estimated to cost at least $500 per day in the lab (22). Given that research often involves working with live organisms or viruses, it is unlikely that the researchers will successfully complete the experiment in the first try. Indeed, there likely will be a lot of failed trials before getting the final results. Therefore, the costs of conducting GOF research are high, and the speed of finishing such studies is slow.

In addition, partially because of the limited materials (like organisms or viruses) needed to conduct GOF research, a study may not have a large sample size and may not satisfyingly answer the research question. The most important materials needed for GOF research are the pathogens. The pathogens usually take a long time to grow successfully, and therefore, the studies most likely do not have a lot of repeated trials. Therefore, the results can be less convincing due to the small sample size. Furthermore, GOF research is only allowed to take place in biosafety level-4 labs, which have the strictest biocontainment and regulations and the highest level of safety and security standards (23, 24). According to the Global BioLabs Report 2023, there are 51 biosafety level-4 labs worldwide. Biosafety level-4 labs experiment with dangerous viruses without available treatments in the field (25). In comparison, there are 157 biosafety level-3 labs in North America, and their less restrictive regulations allow more rapid research progress (26). Considering that GOF research is already inefficient and less productive than other research methods, there have been very few advancements in GOF research and may not satisfy the high demands of rapid life science field development in time (13).

Possibility of Lab Leakage and Deadly Pandemics

The most concerning drawback of GOF research is the possibility of leaking biohazards into the public

and causing deadly pandemics (Fig. 1) (4). Since GOF research aims to study pathogens by enhancing their abilities to transmit or infect organisms, it is possible that there may not be therapeutics available to treat diseases. One study's model simulations show that there is a 5% to 15% probability that the pandemic-possible pathogen leaks out of the laboratory without being detected (27). It is true that these statistics also rely on other factors, including the probability of developing clinical symptoms that the simulations do not account for. However, the results suggest that controllability of escape events is not guaranteed, and laboratories do have possible serious threats to human health.

There are two main causes of the lab leakages. The first one is intentional acting. The most well-known case took place in the early 1970s when Rhodesian military and intelligence services spread a pathogen responsible for cholera in guerrilla camps (28). This event caused more than 1000 people to get infected, and more than 82 people died (29). The second one is accidental acting. There are still rumors going on regarding the source of COVID-19 pandemic (30). One theory is that the pandemic was caused by a lab leakage from a National Institutes of Health (NIH)-funded GOF research in Wuhan, China. Even though there is no solid evidence supporting the theory due to the lack of available data, this possibility still weakens society's confidence in the containment of GOF studies. Even scientists who support continuing GOF research admit that inherent failings of humans cannot be completely prevented (13). The unavoidable "bad actors" possibility, adding on to the probable use of lab results as bioweapons, presents that the risks of GOF research results leaking into the public outweigh the benefits of the research.

Adverse Reactions Brought by Current GOF Treatment Approaches

Adenoviruses are icosahedral and nonenveloped capsids with a double-stranded DNA genome inside (31-34). They are commonly used as vectors to deliver genetic information into host cells. They attracted more attention during COVID-19 pandemic as the Johnson & Johnson coronavirus vaccine utilized non-replicable adenoviral vectors to carry genes for coronavirus surface spike proteins and trigger immunization in hosts (33, 35). The latest generations of adenoviral vectors have most original viral sequences removed and can contain and deliver up to 28 to 32 kb foreign DNA sequences into hosts (31, 32). As a classic example of GOF treatment, it has frequently been used as an effective reason supporting GOF research. However, recently developed assays found that the adenoviral vectors deprived of replication genes would regain replication capability during propagation in host cells, most commonly HEK293 cells (34, 36–40). The more sensitive assays detected several replication-competent adenoviruses in many clinical batches far higher than the current upper limit allowed by regulatory authorities. Past studies showed that even though adenovirus infections usually lead to asymptomatic or mild symptoms in immunocompetent people, the presence of replication-competent adenoviruses was related to increased inflammation, cytotoxicity, prolonged vector clearance, and transgene specific adverse effects (34, 41). Therefore, it is suggested that more studies should be done regarding the adverse reactions caused by use of GOF treatments, and safety towards hosts when using GOF treatments should be reevaluated.

Other Alternatives to GOF Research

There are other research approaches that can obtain similar results as GOF research and are significantly less risky (Fig. 1). One such example is the use of animal models. Due to the rapid development of technology, scientists are trying to modify animal models to present human disease phenotypes. In fact, starting in 2008, there have been animal models developed by independently mutating about 100 genes in mice to successfully show the corresponding human disease phenotypes, including a few types of cancer, heart disease, and neurodegenerative disorder (42). Additionally, there have been other animal models used in scientific research, such as non-human primate models which are considered the most well suited animal models for preclinical testing due to their evolutionary and genetic similarities to humans (43). In the future, more humanized animal models can be developed as well. One successful case is the humanized mouse models. The humanized mice mimic human biology and features by being engrafted with human cells or tissues and expressing human genes. The study of pathogens and diseases that is conducted using GOF experiments can be done by mutating genes in animal models instead. Pathogen-host interactions have been successfully studied in humanized mouse models through successful infection of Salmonella typhi (44). It has been suggested that the use of animal models could replace GOF research as a safer alternative, since animal models could be altered to reproduce the human disease. This has been successfully done with meningococcal disease (45). Another example is the use of loss-of-function research. Loss-of-function analyses modify DNA, RNA, or protein in the pathogens

to reduce certain functions and, in turn, make pathogens less pathogenic (46). Observing the biological behaviors of the pathogens after the change can also elucidate the relationship between genes and corresponding phenotypes. This is a much safer alternative to GOF research as well, since the pathogens are generally less transmissible and virulent after loss-of-function research and less threatening to public health even when accidentally leaked out of the labs.

ARGUMENTS AND OVERLAPS FOR BOTH SIDES

For GOF Research Continuation

There are those arguing for the continuation of GOF research because of its established values – including predicting and developing treatments for future pathogens before they arise - even though most experiments take a long time to show benefits (Fig. 2). The most recent example is the Johnson & Johnson COVID-19 vaccine. Johnson & Johnson-owned Janssen Pharmaceuticals was the third company in the United States to make its coronavirus vaccine available (47). It was the first authorized vaccine that requires only one dose instead of two to maintain high protection against severe disease, hospitalization, and death caused by COVID-19. Johnson & Johnson vaccine can be stored at 4°C, which is the temperature of a regular refrigerator, unlike the Pfizer mRNA vaccines which need to be stored at an ultra-cold temperature of -80°C (48). Even though the Johnson & Johnson vaccine effectiveness was lower than the Pfizer mRNA vaccines upon its release, its protection was stable unlike other existing vaccines at that time that changed significantly. Due to all the benefits shown above, the Johnson & Johnson vaccine was one of the leading vaccines that used a GOF approach to alleviate the limited access to vaccination of the public at that time and greatly contributed to the containment of coronavirus pandemic (49).

The Johnson & Johnson vaccine is a classic example that shows great benefits and values of conducting GOF research (9). Furthermore, biosafety issues are not a primary concern, and people should not be excessively concerned about possible pathogen leakage (13). GOF research is the major method to better understand pathogens and predict and prevent future outbreaks. For those who argue for the continuation of GOF research, their stance is that the decision of halting GOF research will further put the public's health at high risk of facing future deadly outbreaks without any preparation in advance (Fig. 2).

As for the major biosafety concern stated by the opposing side, the supporting side responds that since

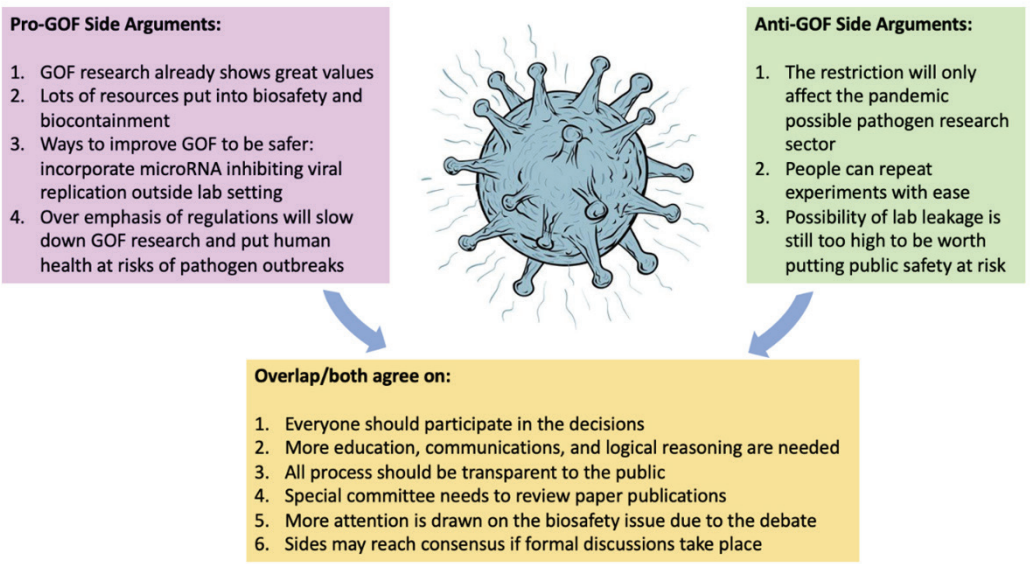

Figure 2. Proponents and Opponents' arguments and overlaps in GOF research debate (13, 50). This figure describes the two sides' arguments and agreements over the future and continuation of GOF research.

the attention was first drawn to GOF research by the H5N1 influenza virus research, a lot of measures have been enforced considering biosafety issues. The GOF research has to take place in biosafety level-4 labs, where daily procedures such as inspection of facilities and equipment and monitoring of disease symptoms are mandated (23). More recently, likely due to the sudden COVID-19 outbreak, US health officials announced that they will enforce stricter guidelines and regulations for GOF research (50). As more and more attention is drawn to potential biosafety issues of GOF research, the likelihood of accidental pathogen leakage from labs is significantly lowered (Fig. 2). Overall, the side supporting the GOF research argues that GOF research often offers details of pathogens that help prevent future outbreaks, and the biosafety concerns of GOF research have been significantly lowered.

For GOF Research Restriction

The side that advocates for halting GOF research argues that due to the quick advance and development in the life science field, the materials and information needed for many experiments can be conveniently accessed or purchased by the public (Fig. 2) (4, 13). Gene editing is one of the common tools used in GOF research. It is now easy for the general public to purchase CRISPR kits or even induced pluripotent stem cells tools to grow stem cells into specialized tissues. Even though it might be expensive to buy such tools, it is possible that individuals or private groups can repeat experiments following the methods mentioned in the papers even without access to the high biosafety level labs. It is impossible to estimate the possibility of people repeating GOF experiments on their own, adding another layer of uncertainty onto the misuse of GOF research (4). Assuming that all GOF research will be conducted with high regulations and biocontainment, there is still a high possibility of accidental and intentional lab leakage (Fig. 2). For those scientists who argue to eliminate GOF research, they state that it is irresponsible to put society at risk when the potential harms of GOF research clearly outweigh the benefits. Moreover, they only want the suspension of pandemic potential pathogens research, which is only a small sector of the complete GOF research category (13). Therefore, the suspension will only affect a small research sector rather than slowing the entire research field (Fig. 2). In summary, the side advocating for GOF research termination is worried about misuse of the research results and emphasizes that the halting will only affect a small section of the life science research field.

Overlaps that Both Sides Agree On

Despite the heated arguments made by both sides in the debate, there are some overlaps that both sides agree on. First, both sides think that people should talk about the GOF research problem face-to-face more in a formal and unbiased manner (13). Currently, too many debates are impacted by press and media intervention and focus on the sensational news. Instead of clearly comparing the logical benefits and harms of GOF research, the problem is presented through an emotional perspective. There is rarely personal interaction between the sides who disagree, so few meaningful debates have occurred (Fig. 2). This is especially ineffective for scientists to try coming up with solutions and rational decisions (13).

Second, the public should all participate in the discussions and decisions regarding whether GOF experiments should be continued (Fig. 2) (13, 51). Currently, most of the logical debates take place within the scientific community, which might be unfair and biased. Many present participants in the scientific community have personal interests at stake since they might be conducting and near finishing an expensive GOF experiment that will be negatively affected by halting of GOF research. Since both GOF research's benefits and harms are closely related to public health, it will only be fair to include people from all different kinds of fields to make decisions. One of the difficulties is to implement inclusion of everyone's opinions since the public usually only gets information from media where selective or partial information is shared for the purpose of framing the debate as a fight to attract attention. From the public's perspective, since the information that they get is far from thorough, and it is strongly emphasized that GOF research can cause possible deadly pandemics, most people are mesmerized into following the trend to argue against GOF research (52). Individuals who are unfamiliar with GOF research are often strongly influenced by extreme perspective pieces on social media or news, creating an overwhelming negative or positive attitude on specific platforms (Fig. 2).

We propose that one effective and fair way to involve the public in the GOF research discussion is to regulate and promote only papers from verified scientists or scientific institutions and include more thorough analysis papers regarding pros and cons of GOF research. This will encourage individuals to make rational decisions based on impartial information. Also, governments should incorporate public opinions in the decision making by randomly picking public representatives from different

professional fields to attend the symposiums. There have been similar approaches enforced (51). The National Academies and the National Science Advisory Board for Biosecurity are main institutional mechanisms, which have held several public meetings aiming to incorporate more public opinions and views regarding GOF research benefits and risks (51, 53, 54). However, the majority of participants had backgrounds in the life sciences and related fields (51). Current literature has indicated that citizens could involve in the discussions through voting, letter, writing, lobbying, and demonstrating. The main factors to fulfill in order to encourage public involvement are abundant education and information from officials to public, collecting opinions from the public through means of surveys or focus groups, and dialogic discussions between citizens and authorities. The paper has also proposed that based on past experiences like the National Academies Symposium, GOF research controversy can be progressed by holding a number of local discussions, followed by a national meeting. Some successful examples in the past were local emergency planning committees and citizen corp councils, where participants interested in community well-being were recruited to join discussions.

Another possible way to impartially educate the public on the topic is to make the process of discussion and approval process of GOF papers more transparent (Fig. 2). This will let the public better understand how the decisions regarding proceeding of GOF research are made and what are the short-term and long-term benefits and risks of such research (55, 56). More transparency will further encourage public engagement and more effective communications between groups. An apparent example is the origin of COVID-19 pandemic. Despite the official announcement that the pandemic did not originate from GOF research lab, there are still many papers trying to find unwarranted evidence of the pandemic conspiracy and accuse the investigation team of not being forthcoming (30). This distrust results from the unclear explanation of the pandemic origin, and further shows that communication and transparency are important to reduce panic and connect the public and the professional fields to unite when facing obstacles.

Both sides of the debate also agree that special committees should be established to review and approve publications of GOF related papers to minimize risks of spreading excessive information that can cause bioterrorism (13). However, under the supervision of other professional researchers and government, the writers and publishers should be primarily responsible for the decisions since they know and are most familiar with the research contents the best (Fig. 2). An example that the GOF research committee can follow is the Recombinant DNA Advisory Committee in the United States. In summary, both sides in the GOF research debate agree that more personal interactions and communications should be made to catalyze the logical decision-making, more people should be involved in the discussion outside scientific community after understanding the complete GOF research issue, the research and publication process should be more transparent, and there should be more regulations regarding the publications of GOF research.

Lastly, it is worth noting that both sides mention the fact that human failings are not fully preventable, but they take different approaches to use this statement for their arguments. For the side encouraging to continue GOF research, they think that the overemphasis of additional rules and regulations will slow down the GOF research that can prevent the public from deadly pathogen outbreaks, which are great threats to human health as well (13). For the side against GOF research, they argue that the unavoidable inherent failings of humans add uncertainty to completely preventing the accidental lab leakage. Therefore, the possibility of lab leakage is still too high to be worth putting public safety at risk. In conclusion, there are many reasonable and opposing arguments made by both sides of the GOF research debate, but they both agree on some details involved in decision-making and publication processes. In addition, it is interesting to see how both sides use the same piece of fact to argue for their different stands. The similarities between the two sides imply that it is possible to reach consensus and agreement regarding regulations of GOF research regardless of their seemingly disparate opinions.

LIMITATIONS & ASSUMPTIONS

There are strong opinions about how the future of GOF research should continue, but there are limitations and assumptions that need to be considered. The main limitation existing in current literature is the dates of the opinion or analysis papers regarding GOF research. Many papers were written before the COVID-19 pandemic, and therefore, their evidence for the arguments might be outdated. There have also been some papers regarding how the pandemic changed the researchers' perspectives on GOF research, which further reflects the great influence of COVID-19 on many people's lines of thinking (57). For example, in a paper published in 2015, the side in favor of GOF research stated that it had been more than a century since the last time there was a major laboratory-

derived human outbreak (13). The side was truly confident about the low possibility of lab leakage incidents causing pandemics. However, there have been prevalent rumors regarding the origin of COVID-19 pandemic. One of the most concerning possible causes was a virus accidentally leaked from a GOF research lab (30). Although this hypothesis was refuted by U.S. intelligence's reported evidence against the theory and still lacked a definite result, the incident affected the supporting side's confidence in the low chance of accidental lab leakage (58).

GOF research conducted before the COVID-19 pandemic was integral to the development of vaccines which decreased the severity of COVID-19 infections (1). Highly efficient vaccines like Johnson & Johnson coronavirus vaccines were quickly developed at the start of the pandemic. Researchers had been working on adenovirus-based vaccines for decades before the pandemic, and when the pandemic broke out, the researchers were able to quickly apply the principles of developing adenovirus-based vaccines to the coronavirus. Johnson & Johnson vaccines could also be stored in refrigerators rather than specialized scientific freezers that were required to refrigerate other vaccines at that time. Along with the fact that one single dose of the vaccine had an efficacy rate of 72 percent in the U.S., the vaccine quickly increased the vaccinated population size and made huge efforts towards the pandemic. To sum it up, the arguments made regarding GOF research before the COVID-19 outbreak should be reanalyzed: the persuasiveness of the unlikelihood of GOF lab leakage is greatly decreased by the "COVID-19 origin conspiracy" hypothesis. The GOF research can indeed speed up vaccine development and help reduce mortality rates of pandemics (1).

More specifically, there are several assumptions made by both sides when they are presenting their arguments. For the side encouraging continuation of GOF research, one most significant argument is that GOF research can better show the interaction between pathogens and hosts, which will aid in disease treatment development (13). However, this benefit assumes that the experiments conducted are successful, and scientists can analyze the results to find useful information. It is true that GOF research can cause potential severe pandemics out of accidental or intentional causes. If the GOF experiments that take a lot of time and costs to be finished turn out to be useless in terms of results, it might not be worth the risks, like possible disease outbreaks and bioterrorism, in the process. Another assumption the side makes is that the professional facilities which uphold BSL standards can be completely relied on in terms of biosafety. They do not take into consideration the possibility of lab workers' intentional lab leakage. Out of 71 high risk human-caused pathogen exposure events from 1975 to 2016, only 76% of the cases are classified as accidental (29). The 17 non-accidental cases are classified as intentional, bioterrorism, and biodefense. One such example is the smallpox epidemic during the French-Indian War in the 18th century (59). The commander of the British forces in North America deliberately provided smallpox-laden blankets for the Native Americans to diminish the hostile population against the British. This intentional use of smallpox as a bioweapon contributed to the smallpox epidemics around the Ohio River Valley for over 200 years. Therefore, from the GOF research perspective, it cannot be guaranteed to produce meaningful results all the time. From the lab facility perspective, the professional workers cannot be completely trusted to handle confidential and deadly pathogens.

As for the side supporting the halting of GOF research, they assume that there are other alternatives that can replace GOF research and produce similarly insightful results (13). However, in reality, the alternatives are not as reliable and effective as GOF research. The first possible alternative is computer modeling. Computer modeling or simulation is designed based on current data on pathogens and infections (60). Information that scientists already know is limited, and therefore, computational methods can only be used as a supplementary tool to predict trends and disease surveillance. They are unlikely to exactly simulate real-world conditions. Another possible alternative is animal models. Even though there are many similarities between animals and humans, results obtained from animal models are not necessarily the same as what is expected to happen in humans. For example, there are slight differences in members of gene families and regulation of gene-expression level, which will translate into physiological differences (61). Physiological variations are especially important for pathogenic disease studies and testing of potential treatments. Overall, GOF research is the most effective method to understand the disease mechanisms and help develop disease treatments, which cannot be replaced by other alternatives.

CONCLUSION

More attention has been drawn to GOF research in the past few decades as the biotechnology field booms with rapid advance and development (62). This paper presents and compares the major benefits and harms of GOF research. On one hand, GOF research can bring

knowledge to scientific communities that no other methods can (13). It allows scientists to know more about pathogens, develop disease treatments, and prevent future pandemics. On the other hand, if the pathogens are leaked into the public, which is unpreventable, the resulting pandemics could be devastating. It is possible that researchers will not have ample time to quickly develop countermeasures against the sudden deadly pandemics, since the pathogens are unnaturally virulent and transmissible. Despite the opposing views the proponents and opponents hold regarding whether to continue GOF research, they both hold the ultimate goal to protect humankind and attempt to minimize harms to society. Public awareness of GOF research has been heightened, especially since the COVID-19 pandemic and thus public and international discourse on GOF research has increased. Individuals against GOF seem to vocalize theories and opinions that have been heavily propagated by social media; theories like GOF research leading to lab leaks have been spread without any scientific validation (63). In response, many countries halted funding for GOF research, which reinforced negative public opinion of GOF research (11). This can be problematic because rapid development of the vaccines like the Johnson & Johnson vaccine were possible because of GOF experimentation. Regardless of being a proponent or an opponent of GOF research, it is undoubted that GOF research is directly tied to public health. Therefore, it is imperative to educate the public thoroughly and objectively about GOF research and encourage all people to participate in decision-making on whether GOF research should be continued. In addition, new papers should be published with updated data and arguments about GOF research, which could modify public opinions. These actions will ensure the legislative regulations and decisions of GOF research are the most beneficial and accepted by the society. Regardless of being a proponent or an opponent in the GOF research debate, the ultimate goal is to minimize public harm.

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DECLARATION OF CONFLICT OF INTERESTS

The author declares that there are no conflicts of interest regarding the publication of this article.

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