Sources:

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https://www.gao.gov/products/gao-23-105455 accessed dec.23.2023

https://www.gao.gov/assets/gao-23-105455.pdf accessed dec.24.2023

https://www.ncbi.nlm.nih.gov/books/NBK285579/ accessed dec.24.2023

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"`Sources im mainly using for my thesis:

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https://www.ncbi.nlm.nih.gov/books/NBK285579/box/box_3-1/?report=objectonly accessed jan.15.2024

Lipsitch M, Galvani AP. Ethical alternatives to experiments with novel potential pandemic pathogens. PLOS Med 2014 May 20;1(5) [**Full text**] accessed jan.28 2024

https://thebulletin.org/2022/06/gain-of-function-research-cant-deliver-pandemic-predictions-are-there-alternatives/ accessed jan.28.2024

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https://medium.com/@queenskisivuli/randomness-in-computer-science-the-good-the-bad-and-the-ugly-f47c26258585 accessed feb.4.2024

https://theconversation.com/gain-of-function-research-is-more-than-just-tweaking-risky-viruses-its-a-routine-and-essential-tool-in-all-biology-research-202084 accessed feb.3.2024

https://www.criver.com/eureka/what-bsl-3-lab accessed.mar.8.2024

Framework for GOF research:
https://www.phe.gov/s3/dualuse/Documents/funding-hpai-h5n1.pdf

Platform access: https://platform.cysf.org/project/edit/attachments/

- Gain-of-fucntion (GOF) is used in many different ways but is mainly used for making pathogens more infectious to humans and is used to understand the genetic makeup of an organism
- Enhanced Potential Pandemic Pathogens (ePPPs) are a type of GOF engineered to become more fatal or more contagious
- GOF and Loss-of-function (LOF) are basically a bit like the opposite of each other. While GOF focuses on giving pathogens more abilities, LOF tries to weaken the pathogen and to break it down(?)
- Although, both methods are used to understand the genetic makeup of the pathogen so that we can develop more, vaccines, therapies, cures, and stuff of the sorts for those certain pathogens
- GOF is widely a controversial topic/technique of pathogen research because,

1. GOF is really risky if a lab leak were to happen. Because, we humans make mistakes because, we're human. Sometimes mistakes are unavoidable.

(Continued on Dec.24)

- "The U.S. government has released the *U.S. Government Gain-of-Function Deliberative Process and Research Funding Pause on Selected Gain-of-FunctionResearch Involving Influenza, MERS, and SARS Viruses in October 2014* as a sort of guide to GOF researched and has put a pause to 14 federally funded research products but in the end 7 had an exemption from this"
- ePPPs are any microorganism that are highly transmissible across humans and can cause an uncontrollable spread. They are also highly virulent and can cause high morbidity and/or mortality among humans, and has the potential to cause a pandemic, or have caused a pandemic.
- Examples: include the H5N1 influenza viruses(link is external), also referred to as bird or avian influenza, SARS-CoV(link is external), which caused an epidemic in several countries in 2003, and SARS-CoV-2(link is external), also known as Severe Acute Respiratory Syndrome Coronavirus 2, which causes COVID-19 disease. Genetic changes or mutations in pathogens, especially viruses that have ribonucleic acid as its genetic material, regularly occur in nature. Some mutations in nature can cause pathogens to gain new functions or enhance existing characteristics such as fitness or pathogenicity (ability to cause disease) as has been seen with the many variants of SARS-CoV-2 since the beginning of the pandemic.

(Jan.1)

- GOF research helps us understand a pathogen and how to battle it if it does manage to mutate and evolve
- More safety measures have to be implemented in order to protect the public from these pathogens if it does manage to leak
- There is a thing called “The Framework” that sort of defines what an ePPP is
- In recent years has caused a lot of controversy among people due to its possibly deadly repercussions if mistakes are made

(Jan.4)

- GOF research is done through a genetic mutation in the organism

(Jan.7)

- People have expressed the term GOF as too broad.

[]“Many participants pointed out during the course of the meeting that the broad term “gain-of-function” needs some refinement that will differentiate the type of experiments typically performed for basic virological research from experiments that clearly raise concerns. When asked to define where virological research crosses the line into GoF research as defined by the U.S. government (White House, 2014a), Subbarao responded that “the term gain-of-function is used by geneticists and is a vague and unsatisfactory term for microbiologists.” This statement was echoed by Imperiale and many others during the discussion.” - https://www.ncbi.nlm.nih.gov/books/NBK285579/

- GOF alternatives?
- Although, these alternatives may not answer all the key questions that virologists have for certain pathogens, or, may lead to misinformation and inaccurate information

(Jan.11)

- Science fair meeting in the construction lab
- Should work on my conclusion for the problem

(Jan.13)

- Hypothesis
- Plan out trifold

Hypothesis: I predict that yes, ePPP research should totally be monitored heavily. But, it is still necessary because even if there are different alternatives to GOF research, they are either deemed inaccurate or are simply not enough to answer the questions scientists ask.

- Started working on the research, and problem part.

(Jan. 14)

- Started work on my thesis
- Gonna ask help from my brother to help me format this information.

(Jan. 14)

- Started working on thesis

(Jan. 15)

- Working on thesis
- Plan out presentation
- Reading up on this link (https://www.ncbi.nlm.nih.gov/books/NBK285579/) to support my thesis
- []“[Dr. Kanta] Subbarao emphasized that current medical countermeasures are often insufficient largely because of resistance mechanisms that lead to “escape mutants,” that is, drug-resistant strains. There is, therefore, a continual need to develop new antiviral drugs and additional options, such as immunotherapy, based on neutralizing monoclonal antibodies. Ultimately, GoF studies, which enhance viral yield and immunogenicity, are required for vaccine development. Molecular methods help with the characterization of antigenic variants, elucidate the biological basis for adverse outcomes associated with vaccine candidates, and determine the basis for attenuation and stability of vaccine candidates.” - https://www.ncbi.nlm.nih.gov/books/NBK285579/
- [1] “General Virology Questions and Questions Specific to Influenza, SARS, and MERS Research
- Why/how does the virus infect and kill mammals?
    - What are the critical host range and virulence determinants of MERS-CoV?
    - Why are some influenza strains more virulent than others?
- Do antiviral drugs work, and how does the virus become resistant?
    - Can we identify antiviral drugs that are safe and effective for MERS-/SARS-CoV?
    - What drives the evolution of influenza antigenic change and antiviral resistance?
- Do current or novel vaccines or monoclonal antibodies provide protection, and can the virus escape?
    - Can we develop a SARS-/MERS-CoV candidate vaccine that is safe, immunogenic, and efficacious?
    - Can monoclonal antibodies be used safely for prevention and treatment?
    - Are there some influenza viral targets that will not allow escape from the immune system?
- How does the virus spread within animals or between animals?
    - Why do some influenza strains spread efficiently while others do not?
- Could the virus cause a pandemic?
- What is the likelihood of (re)emergence?
    - Will SARS or a SARS-like CoV re-emerge from bats or other animal hosts?”

SOURCE: [Dr. Kanta] Subbarao's list of general and influenza/SARS/MERS specific questions in virology, symposium presentation, 2014.

- Put the citation for thesis as: Board on Life Sciences; Division on Earth and Life Studies; Committee on Science, Technology, and Law; Policy and Global Affairs; Board on Health Sciences Policy; National Research Council; Institute of Medicine. Potential Risks and Benefits of Gain-of-Function Research: Summary of a Workshop. Washington (DC): National Academies Press (US); 2015 Apr 13. 3, Gain-of-Function Research: Background and Alternatives. Available from: https://www.ncbi.nlm.nih.gov/books/NBK285579/
- [3] 'Subbarao emphasized that current medical countermeasures are often insufficient largely because of resistance mechanisms that lead to "escape mutants," that is, drug-resistant strains. There is, therefore, a continual need to develop new antiviral drugs and additional options, such as immunotherapy, based on neutralizing monoclonal antibodies. Ultimately, GoF studies, which enhance viral yield and immunogenicity, are required for vaccine development. Molecular methods help with the characterization of antigenic variants, elucidate the biological basis for adverse outcomes associated with vaccine candidates, and determine the basis for attenuation and stability of vaccine candidates.'
- [4] "The essence of the debate around the risks and benefits of GoF research and the concerns it raises have naturally encouraged virologists on both sides of the debate to consider alternative methodological approaches. During his talk, Kawaoka discussed alternatives to GoF research mostly applicable to influenza research, such as loss-of-function research, use of low pathogenicity viruses, and phenotypic analyses. He further cited a review paper in which Lipsitch and Galvani (2014) stated that "alternative scientific approaches are not only less risky, but also more likely to generate results that can be readily translated into public health benefits." However, Kawaoka argued through specific examples that alternatives do not always provide the full answer to key questions. For instance, he cited work by Tumpey et al. (2007) and Imai et al. (2012) on mutations responsible for the loss of transmission capabilities of the 1918 influenza strain between ferrets and noted that this work required GoF research because a loss-of-function approach did not provide the complete picture. In addition, although working with low pathogenic avian influenza viruses provides a safer approach, Kawaoka explained that "highly pathogenic avian influenza differ from low pathogenic viruses in their kinetics of virus replication and tropism" and therefore the data can be misleading. Other alternatives discussed by Kawaoka and Dr. Robert Lamb, Northwestern University, in Session 8 of the symposium were cited from the recent review paper by Lipsitch and Galvani (Box 3.3). Kawaoka concluded that even if these approaches offer safer alternatives to GoF research of concern, for some questions researchers cannot rely solely on them because the phenotype of and the molecular

basis for these new traits have been identified by GoF research but not by alternative approaches.”

- [5] “Alternative Research Methods with Potentially Less Risk
- Molecular dynamical modeling of influenza proteins and interactions with inhibitors and receptor
- In vitro studies of specific properties required for human adaptation, using single proteins
- In vitro studies of genetic interactions between loci in one or several viral proteins using replication-incompetent viruses – epistatic interactions
- Sequence database comparisons of genetic properties of human and avian adapted viruses
- Comparisons of human seasonal isolates and zoonotic isolates from infected humans and avian isolates”

SOURCE: Lipsitch and Galvani, PLoS Med included in Kawaoka's symposium presentation, 2014.”

- Alternatives have been proposed in vivo models rather than the process itself

(Jan.21)

- working on thesis

(Jan.22)

- still working on thesis… （◞‸◟）

(Jan.23)

- Looking over the information I put in the science fair website

(Jan.28)

- Looking over data
- Decided to finish up thesis
- Trying to research on possible alternative approaches to GOF research
- GOF research has been a very divided topic among scientists
- 2 main reasons why GOF research is done: To detect dangerous viruses early, and to help vaccined prevent more dangerous strains of pathogens (although Lipsitch M,

Galvani says that people who have developed vaccines denies that GOF research even helps them???)

- Very differing things on both sides of the topic. While GOF advocates say that alternatives are probably less informative, the opposing side says that alternatives could be more informative which is kind of confusing
- Lipsitch proposes that
- “Some approaches start with sequence analysis and molecular dynamics modeling, which are intrinsically safe. The experimental evaluation of hypotheses raised by such studies may use viral components rather than the entire infectious virus, making these experiments simultaneously safer and more precise and mechanistic than engineering PPPs. Furthermore, these approaches are typically less costly than PPP experimentation, facilitating phenotypic evaluation of a greater diversity and abundance of genetic variants. Ultimately, studies with intact viruses will be necessary for a full understanding of human transmissibility, a phenotype of a whole virus. Elucidating the evolutionary trajectory through which existing seasonal (former pandemic) viruses became transmissible from avian precursors is safer than PPP experimentation, given. that there is preexisting population immunity to seasonal strains, the products of such evolution.” lipsitch”s article
- “For avian influenza viruses or bat coronaviruses, scientists can engineer mutations in isolated genes for key proteins on the viral surface (hemagglutinin for influenza and spike for coronaviruses) and generate viral pseudotypes. This basically means grafting the surface proteins onto a virus with a harmless track record in humans. Through this method, researchers can identify a catalogue of mutations that can help the pseudotypes infect human cells. Another method involves using single-cycle virus derivatives—viruses harboring a genetic lesion that limits them to a single round of growth. The single-cycle derivatives allow researchers to identify mutations in genes other than hemagglutinin or spike that might help the virus adapt to a new host cell.” (https://thebulletin.org/2022/06/gain-of-function-research-cant-deliver-pandemic-predictions-are-there-alternatives/)
- Another big reason why GOF research is done is to “predict” potential upcoming pandemics which is probably almost impossible because 1 mutation in a virus could lead to many, many outcomes
- The term GOF was coined around the early 2000s when the H5N1 avian influenza and 2 labs decided to conduct research on them. Resulting in genetically modified strains of the H5N1 influenza virus was created. But, before the labs could publish their research, they were halted by the US National Science Advisory Board for Biosecurity, halted and examined the reports before they could be released to the public because they were afraid that if too much information was released on that report, that bioterrorists could use that information and make their own PPPs and unleash it onto normal people.
- “In genetics, “gain of function” usually refers to a mutation that results in an enhanced phenotype compared with the wild-type allele. For example, a mutation that increases metabolism of a substrate by a factor of 2 would be considered a gain of function. From a technical standpoint, nearly all microbial evolution work could therefore be considered “gain of function” because any experiment that forces a microbial population to evolve

could result in a pathogen with new or enhanced characteristics, which may increase a pathogen's fitness, virulence, or transmissibility." - https://www.embopress.org/doi/full/10.15252/embr.202153739

(feb.3.2024)

- "Exploring less risky alternatives to GOFR is essential. Options such as using low-pathogenic strains or focusing on loss-of-function research have been considered, but they have limitations. Low-pathogenic strains may not accurately replicate target pathogens, while loss-of-function research alone may not provide sufficient data. Researchers should also explore other safer alternatives, such as studying single proteins or replication-incompetent viruses in vitro, utilizing in silico modeling and conducting long-term genetic sequence comparisons. These alternatives offer valuable insights without the risks associated with enhanced virulence." https://www.cola.org/striking-a-balance-navigating-the-risks-and-rewards-of-gain-of-function-research-part-3/
- General framework/criteria for GOF research
    - 1) The virus anticipated to be generated could be produced through a natural evolutionary process;

      2) The research addresses a scientific question with high significance to public health;

      3) There are no feasible alternative methods to address the same scientific question in a manner that poses less risk than does the proposed approach;

      4) Biosafety risks to laboratory workers and the public can be sufficiently mitigated and managed;

      5) Biosecurity risks can be sufficiently mitigated and managed;

      6) The research information is anticipated to be broadly shared in order to realize its potential benefits to global health; and

      7) The research will be supported through funding mechanisms that facilitate appropriate oversight of the conduct and communication of the research.

      If a proposal meets these criteria and is being contemplated for funding, the agency will submit the proposal for Department-level review. The Department-level review will provide multidisciplinary expertise—including public health, scientific, security, intelligence, countermeasures, and preparedness and response—to evaluate these proposals. The Department-level review will also identify any additional risk mitigation measures that are required, and determine whether a given proposal is acceptable for HHS funding. For proposals that are deemed acceptable for HHS funding, the funding agency within HHS will make the final funding decision. Proposals that have been determined to be

unacceptable for HHS funding through Department-level review are not eligible for funding agency support. Figure 1 outlines the review process described by the Framework.

The U.S. Government Policy for Oversight of Life Science Dual Use Research of Concern (March 29, 2012) defines dual use research of concern as "life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security." 2 HPAI H5N1 viruses are defined here as influenza viruses that express the virulent form of the hemagglutinin (HA) gene from highly pathogenic H5N1 virus. 3 Proposals aimed at characterizing naturally occurring strains are exempt from this Framework.

https://www.phe.gov/s3/dualuse/Documents/funding-hpai-h5n1.pdf

- Maybe we can use computer simulations for a alternative but that's too far-fetched
- If someone were actually to try to develop a software, I think it'd take years and years to achieve the randmoness needed. Also, computers aren't truly random. Plus, if someone else used the program and found out the code, they could easily find out the outcomes. So, computer generation is off the cart for now.
- Mostly finished online part
- Planning out trifold
- Starting presentation

(Feb. 11.2024)

- Working on presentation and condensing the info
- Polishing the online portion

(March 7.2024)

- SF meeting

(March 8)

- Working on finishing presentation details and video

(mar.9.2024)

- Recording vid and made script

(mar.9 -- april 12)

- Will start working on trifold
- Maybe a school trial science fair?