Case Studies of HPAI H5N1 Gain-of-Function Research

Introduction: The following case studies are intended to exemplify a range of HPAI H5N1 gain of function research proposals and to serve as a tool for exercising the proposed HHS framework for guiding funding decisions about such research. This framework applies to research projects that fall within the scope described in text box 1 below.

Box 1. Applicability of the proposed framework for guiding HHS funding decisions about HPAI H5N1 gain-of-function research

HHS will apply this review framework to proposals that are reasonably anticipated to confer gain-of-function attributes to influenza viruses expressing the virulent form of the hemagglutinin (HA) gene from highly pathogenic H5N1.

In order to be considered acceptable for HHS funding, HPAI H5N1 gain of function research projects that fall within the scope of this framework must fulfill all of the criteria listed in text box 2 below.

Box 2. Criteria for guiding HHS funding decisions for HPAI H5N1 gain-of-function research proposals

HPAI H5N1 gain-of-function research proposals are acceptable for HHS funding only if:

1. The research addresses a scientific question with high significance to public health;
2. The research does not intend, nor is reasonably anticipated to yield an HPAI H5N1 experimental virus that has increased transmissibility, pathogenicity, or expanded host range, unless there is evidence that such a virus could be produced through a natural evolutionary process in the foreseeable future;
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 an HPAI H5N1 research proposal is in accord with all of the criteria in Box 2, and the HHS funding agency is considering funding the proposal, additional Department-level HHS review is required if the proposal exhibits any of the characteristics in Box 3 to determine if the proposal is acceptable for HHS funding.

Box 3. Characteristics of HPAI H5N1 gain-of-function research proposals that warrant Department-level HHS review

HPAI H5N1 gain-of-function research proposals that meet the criteria in Box 2, and that the HHS funding agency is considering funding, require Department-level review if the studies intend or are reasonably anticipated to:

- Increase pathogenicity, virulence, and/or transmissibility of a virus in mammals; or
- Disrupt the induction of a host's innate immunity; or
- Interfere with the effectiveness of an available vaccine; or
- Confer to the agent resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent; or
- Facilitate the virus' ability to evade detection methodologies.

CASE STUDY A: In this experiment, the researchers aim to engineer and characterize highly pathogenic H5N1 viruses that are transmissible by respiratory droplets in two mammalian model organisms: ferrets and non-human primates. The researchers propose experiments that serially passage in ferrets an HPAI H5N1 virus that has been demonstrated to be transmissible by respiratory droplets in ferrets to further increase pathogenicity while retaining its transmissibility. They aim to identify mutations throughout the genome that will enhance virulence of a virus while retaining its transmissibility via respiratory droplets in ferrets. Viruses with enhanced virulence that are transmissible by respiratory droplets between ferrets will then be tested for pathogenicity and transmissibility in non-human primates.

1. **Aim:** To understand the molecular relationship between pathogenesis and transmissibility by identifying how pathogenic HPAI H5N1 viruses can become while maintaining efficient transmissibility by respiratory droplets in ferrets.
2. **Assays and model systems:**
   a. ***In vitro*:** Reverse genetics to generate mutant HPAI H5N1 viruses possessing mutations previously demonstrated to confer respiratory droplet transmission in ferrets.
   b. ***In vivo*:** Serial passaging of viruses that are transmissible in ferrets to select for HPAI H5N1 viruses that are more virulent than the starting virus and retain transmissibility by respiratory droplets in i) ferrets and ii) non-human primates.
3. **Starting strains:** HPAI H5N1 viruses, engineered to include mutations that increase pathogenicity and/or enable transmission by respiratory droplets between ferrets.
4. **Anticipated results:** Generation of HPAI H5N1 viruses that are transmissible by respiratory droplets between ferrets and have been passaged in ferrets to determine if pathogenic viruses arise in the ferret model. They will also potentially generate viruses that are highly pathogenic and transmissible between non-human primates (possibly by respiratory droplets).
5. **Biosafety conditions:** BSL-3 with enhancements required for work with HPAI viruses.
6. **Countermeasure efficacy:** Anti-virals and vaccines may be effective against the starting strains; unknown whether they will be effective against resulting strains but this can be evaluated experimentally.

CASE STUDY B: In this experiment, investigators are interested in further exploring the mechanisms that control HPAI H5N1 virus transmissibility in the ferret model. To gain a better understanding of the mutations and functional changes in the H5 HA gene that result in transmissible viruses, the researchers will determine whether genetic changes shown to confer efficient respiratory droplet transmission in

ferrets for clade 1 or 2.1.3 viruses also confer similar properties on H5N1 viruses from other currently circulating clades and subclades. Where needed, additional or different mutations will be introduced into viruses to attempt to achieve respiratory droplet transmission in the ferret model.

1. **Aim:** To examine whether HPAI H5N1 virus transmissibility in mammals is achieved through similar genetic changes in different HPAI H5N1 clades and subclades.
2. **Assays and model systems:**
    a. ***In vitro*:** Reverse genetics to generate mutant HPAI H5N1 viruses representing different H5N1 clades and subclades possessing mutations previously demonstrated to confer respiratory droplet transmission in ferrets and other mutations as identified by sequence analyses.
    a. ***In vivo*:** Analysis of mammalian transmissibility by inoculating ferrets (intratracheally and intranasally) with naturally-occurring and engineered HPAI H5N1 viruses; analysis of whether the H5N1 viruses are transmissible by respiratory droplets in ferrets.
3. **Starting strains:** Viruses generated by reverse genetics chosen to represent naturally-occurring HPAI H5N1 viruses from several clades
4. **Anticipated results:** Investigators will engineer and characterize H5N1 viruses from previously untested clades and subclades to determine if mutations identified previously for clade 1 and clade 2.1.3 viruses confer respiratory droplet transmission in the ferret model and determine if these viruses have altered pathogenicity in ferrets. The investigators may identify HPAI H5N1 viruses (or mutations) that are highly pathogenic and transmissible by respiratory droplets in ferrets.
5. **Biosafety conditions:** BSL-3 with enhancements required for work with HPAI viruses.
6. **Countermeasure efficacy:** Anti-virals and vaccines may be effective against some of the starting H5N1 viruses. It is unknown if they will be effective against the resulting strains but this can be evaluated experimentally.

CASE STUDY C: Previous experiments have shown swine to play an important role in influenza ecology by serving as a "mixing vessel" for influenza viruses. To assess the potential of HPAI H5N1 viruses to reassort with human influenza viruses in nature, investigators propose to co-infect swine with various wild-type HPAI H5N1 and human influenza viruses. Viruses will be recovered from the co-infected swine and genotyped, and reassortant viruses will be tested along with parental viruses for pathogenicity and respiratory droplet transmissibility in the ferret model.

1. **Aim:** To test whether wild-type HPAI H5N1 and human influenza viruses can reassort in swine hosts.
2. **Assays and model systems:**
    a. ***In vivo*:** Generation of reassortant viruses by co-inoculation of swine with HPAI H5N1 and human influenza viruses; characterization of pathogenicity and transmissibility of reassortant viruses in ferrets.
3. **Starting strains:** Naturally-occurring HPAI H5N1 viruses and human influenza viruses.
4. **Anticipated results:** Generation of HPAI H5N1 and human influenza reassortant viruses. It can be anticipated that the investigators will identify and characterize reassortant viruses that are transmissible in ferrets (possibly by respiratory droplets).
5. **Biosafety conditions:** BSL-3Ag (swine co-infections); BSL-3 with enhancements required for work with HPAI viruses (ferret studies)
6. **Countermeasure efficacy:** Anti-virals and vaccines may be effective against some of the starting H5N1 and human influenza viruses. It is unknown if they will be effective against the resulting strains but this can be evaluated experimentally.

CASE STUDY D: Researchers have identified mutations that confer virulence to HPAI H5N1 in the ferret model. These mutant viruses, though transmissible in ferrets, were not readily transmissible in non-human primates. To test the significance of these mutations and study the host response in non-human primates, the investigators propose cloning relevant portions of the HPAI H5N1 genome (containing the identified mutations) into another viral vector known to readily infect the human respiratory tract.

1. **Aim:** To test the virulence in non-human primates of an engineered HPAI H5N1 virus using a viral vector delivery system that targets the virus to the respiratory tract.
2. **Assays and model systems:**
    a. ***In vitro*:** Reverse genetics to generate mutant HPAI H5N1 viruses possessing mutations previously demonstrated to confer virulence and respiratory droplet transmission in ferrets; constructing a viral vector delivery system containing the engineered virulent components of HPAI H5N1.
    b. ***In vivo*:** Targeted delivery of the engineered viral components to the respiratory tract of non-human primates.
3. **Starting strains:** HPAI H5N1 viruses, engineered to include mutations that increase virulence in ferrets; viral vector known to readily infect the human respiratory tract.
4. **Anticipated results:** Generation of a hybrid virus that contains virulence factors from HPAI H5N1 and is readily targeted to the respiratory tract of non-human primates, and is therefore potentially transmissible (perhaps by respiratory droplets). By using a viral vector that is known to infect the human respiratory tract, this study may generate a highly pathogenic hybrid virus that is transmissible in respiratory droplets in humans.
5. **Biosafety conditions:** BSL-3 with enhancements required for work with HPAI viruses.
6. **Countermeasure efficacy:** Anti-virals and vaccines may be effective against the starting strains; unknown whether they will be effective against resulting strains but this can be evaluated experimentally.