

## **The current state of H5N1 vaccines and the use of the ferret model for influenza therapeutic and prophylactic development**

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### **Abstract**

Highly pathogenic avian influenza H5N1 is a threat to global public health as a natural pandemic causing agent but has recently been considered a bioterrorism concern. The evolving view of the H5N1 virus necessitates the re-evaluation of the current status of H5N1 therapeutics and prophylactics, in particular the preparation of viable H5N1 vaccination strategies as well as the use of ferrets in influenza research. Here the highly pathogenic H5N1 virus dilemma is discussed in context with the current H5N1 vaccine status and the use of the ferret model. Previously, the development of various H5N1 vaccine platforms have been attempted, many of them tested in the ferret model, including vector vaccines, adjuvant vaccines, DNA vaccines, and reverse engineered vaccines. Moreover, as ferrets are a superlative animal model for influenza investigation and vaccine testing, it is imperative that this model is recognized for its uses in prophylactic development and not only as an agent for creating transmissible influenza viruses. Elucidating the ferret immune response and creating ferret immune reagents remain important goals in conjunction with the development and manufacture of H5N1 vaccines. In summary, an efficacious H5N1 vaccine is urgently needed and the ferret model remains an appropriate model for its development.

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Highly pathogenic avian influenza H5N1 (HPAI H5N1) is a threat to global public health as a natural pandemic causing agent but recently there has been a great deal of controversy surrounding the possibility of H5N1 as a biosecurity or bioterrorism risk [1,2]. As a natural pandemic threat, an approximately 60% mortality rate has been calculated for HPAI H5N1 virus in infected humans [3]. Furthermore, if this virus acquired human-to-human transmission capability, it is predicted that an unprecedented lethal pandemic would possibly ensue in the global naïve human population. Recently, the laboratory development of an aerosol transmissible H5N1 virus has been published [4] and a similar study is in waiting. These studies have ignited a serious debate on whether an aerosol transmissible H5N1 virus can be utilized for bioterrorist objectives or are a biosecurity danger [1,5–7]. It is important to recognize that the scientific aim and rationale of these investigations was to determine the capability of HPAI H5N1 to acquire aerosol transmission and to elucidate possible mechanisms of aerosol transmission in the ferret model, a natural influenza host. The researchers contend that these findings will

contribute significantly to H5N1 virus therapeutic and prophylactic development[5] .

The results of these H5N1 ferret transmission studies represent the second facet of concern for H5N1 as a biosecurity and bioterrorism hazard which has been heavily debated [1,5–7]. The publicity that was generated from this work prompted a 60-day international hold on H5N1 influenza transmission research. Furthermore, this controversy sparked a call for redaction of the full results by the United States National Science Advisory Board for Biosecurity (NSABB) in these soon-to-be published papers[5,6]. The NSABB labeled the technologies established in these seminal studies as “dual use research of concern,” *i.e.*, research having the potential to be used for good or bad purposes [1,5,7]. With this possibility in mind, HPAI H5N1 not only remains a highly virulent natural pandemic threat, but the deliberate misuse of a human developed aerosol transmissible H5N1 virus is a concern. With controversy surrounding the H5N1 virus and its natural pandemic causing potential, it is now more important than ever to discuss and evaluate the timely

development of H5N1 therapeutics and prophylactics, including the preparation and efficacy of viable H5N1 vaccination strategies.

Estimates place the financial burden of a highly virulent pandemic to be in the neighborhood of 200 billion US dollars in the United States alone [2]. The ideal strategy in combating an eminent pandemic is producing pre-breakout prepared influenza vaccines, which further implies the need for immediate vaccine strategy development. Previously, researchers have attempted to create HPAI H5N1 vaccine candidates with non-pathogenic yet antigenically related non-pathogenic viruses; however, these attempts have had little success [8].

Currently, various H5N1 vaccines are being developed including vector vaccines, adjuvant vaccines, DNA vaccines, and reverse engineered vaccines, although there are several points to consider for their development. Controversy exists around the method of vaccine type and vaccine production. The US stockpile of H5N1 vaccine to A/Viet Nam/1194/2004 is propagated in embryonated chicken eggs despite the problems that are associated with the use of chicken eggs in the event of a pandemic avian virus [9,10]. Dangers in the use of chicken eggs as well as the availability of chicken eggs are major concerns in an avian derived influenza pandemic [11]. Furthermore, the World Health Organization promotes cell-based H5N1 influenza vaccine technologies using either Vero or MDCK cells and efforts are being made to investigate the production of cell-based H5N1 vaccines [10,12,13]. Other issues to consider with the development of an HPAI H5N1 vaccine include the propensity of genetic drift and shift of the influenza virus. As genetic drift and shift of influenza viruses may result in antigenically distinct viruses, a shifted or drifted HPAI H5N1 may render a previously manufactured vaccine ineffective.

Presently in the US, two types of broad influenza strain egg/cell-derived vaccine platforms are approved for clinical use: live attenuated influenza vaccines (LAIVs) and inactivated influenza vaccines (whole/subvirion) [2,14]. Examples of inactivated influenza vaccines include the current 2011-2012 seasonal trivalent influenza vaccine FluLaval produced by GlaxoSmithKline (Brentford, UK) [15]. FluLaval is a composition of three heat-inactivated influenza viruses recommended by the WHO:

A/California/7/2009 (H1N1)-like virus, an A/Perth/16/2009 (H3N2)-like virus, and a B/Brisbane/60/2008-like virus (B Victoria lineage)[15,16].

LAIVs have various advantages over the inactive virus vaccines including a greater immune response and efficacy [2]. LAIVs are often developed by cold-adaptation, influenza virus reassortants and/or genetically modified influenza virus technology. Importantly, the reverse engineered virus platform is a conglomerate of a basic non-pathogenic backbone of an innocuous influenza virus and the immunogenic proteins of the pathogenic influenza strain of interest. Currently, the non-pathogenic backbone of the cold-adapted, live attenuated viruses is the FDA-approved LAIV against seasonal influenza strain (A/AnnArbor/6/1960CaH2N2), which easily grows to high titres for mass production at cold temperatures [2]. Furthermore, the H5N1 rg vaccine contains the immunogenic proteins from pathogenic H5N1 viruses and the nonpathogenic backbone proteins from the A/PR/8/1934 virus [10]. Although concerns exist over the use of cold-adapted LAIVs, several studies have shown these vaccines have been effective and safe [17–19].

As non-pathogenic antigenically related native H5N1 virus vaccines have had little success, efforts have been made in the development of an H5N1 LAIV using reverse genetic techniques with cold adapted viruses [16]. Previously, a polybasic amino acid region at the HA cleavage site has been identified as a key region responsible for H5N1 pathogenicity [20,21]. Importantly, this identified polybasic aa section adjacent to the cleavage site can be manipulated to create a virus of less pathogenicity for the use in LAIV derivation [10,21,22]. Investigations of reverse engineered H5N1 vaccines for rg-A/Vietnam/1203/2004, rg-A/Indonesia/05/2005 and rg-A/Anhui/1/2005 have been completed [23–25]. Although these vaccines did not show the hoped efficacy [16;21], this vaccination platform represents an important route for further investigation.

With the current controversy surrounding the use of ferrets in influenza studies, it is important to remember that the reason for using ferrets extends far beyond that of providing a model for H5N1 transmission, as many investigators have shown ferrets to be a superlative platform for testing and

developing influenza therapeutics including the investigation of influenza vaccines [26–30]. Importantly, when infected with respiratory viruses, ferrets display many of the symptoms and pathological features seen in infected humans [31–33]. Ferret immune responses have been shown to mimic the immune response of humans, which makes the ferret an appropriate tool for the investigation of human diseases, including H5N1. Furthermore, each influenza strain and subtype is characterized by a unique set of clinical features and innate and adaptive immunological signatures following infection with pandemic H1N1, seasonal H1N1, seasonal H3N2, and influenza B viruses [34]. As well, the characterization of ferret cytokines and immune molecules, essential components to the evaluation of therapeutics and the immune response during pathogenic virus have been previously described [35–37]. Currently, ferrets are used for influenza drug testing; for example, neuraminidase inhibitors are effective during ferret influenza infection [38–40]. Importantly, ferrets display immunological memory and cross-protective immunity, the cornerstones of vaccine mechanism, and are therefore ideal for the use of testing the safety and efficacy of human vaccines [41–45].

The diverse and broad antigenicity of H5N1 isolates renders it very difficult to accurately predict which types of vaccines would be effective against a potential H5N1 pandemic. The ferret is also an ideal model for testing a variety of experimental vaccines and their effectiveness in reducing illness caused by infections by candidate H5N1 strains. Moreover, due to its natural susceptibility to human influenza, the ferret is used extensively to generate hyperimmune sera against seasonal influenza viruses. As well, the use of reverse genetics has enabled the production of antisera against highly pathogenic influenza viruses without causing severe illness to the animals [46]. If an outbreak of H5N1 were to occur in humans either by natural exposure or through a laboratory release, ferret H5N1 virus- and vaccine-specific antisera will be critical for both the identification of infectious virus strains and the rapid prediction of which vaccines will be effective.

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