[redacted]

# Form
## Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

## 1 General information

| | | |
|---|---|---|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. | [redacted] |
| 1.2 | Provide the name of the licenced establishment. | Biomedical Primate Research Centre |
| 1.3 | Provide the title of the project. | Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza infection in macaques |

## 2 Categories

2.1 Please tick each of the following boxes that applies to your project.

- ☐ Basic research
- ☒ Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or animal
- ☐ Research aimed at preserving the species subjected to procedures
- ☐ Higher education or training
- ☐ Forensic enquiries
- ☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

## 3 General description of the project

**3.1 Background**

Describe the project (motivation, background and context) with respect to the categories selected in 2.1.

Influenza epidemics are estimated to result in infection of 2,5-10% of the world population every year, causing 2-5 million cases of severe illness and 250.000-500.000 deaths (1). Vaccination is considered the

most effective measure against the influenza disease and, as such, it is recommended by the European Council (2) and implemented in all EU/European Economic Area member states. The main problems of the current influenza vaccines are; a) they are not very effective in the elderly, b) they only protect against highly homologous strains, while circulating influenza virus strains constantly evolve as a result of antigenic drift, c) they do not protect against new pandemic strains that emerge as a result of recombination between different viral strains found in animal reservoirs, d) they do not protect against highly pathogenic avian influenza virus (3-5). These problems are amplified by the cumbersome current production methods, which involves growing the virus on eggs to prepare inactivated- or live attenuated- influenza vaccines. The 6 months required for vaccine manufacture means that the vaccines have to be based on predictions about which virus strains will circulate during the next influenza season. A mismatch between the vaccine and the actually circulating influenza strain(s) however, results in lower vaccine effectiveness as shown for the 2014-15 influenza season with regard to the H3N2 strain (6). New vaccine strategies that can provide broader protection and cover a range of seasonal influenza strains as well as pandemic and avian influenza virus strains are urgently needed (7). These so called "universal" influenza vaccines are directed at either a) inducing broadly neutralizing antibodies by targeting the relatively conserved stem region of the haemagglutinin (HA) subunit, which is responsible for virus entry into the target cell, b) induction of antibodies to the neuraminidase (NA) surface glycoprotein (8), c) inducing protective T-cell responses that are usually directed against more conserved proteins of the virus and therefore provide broad recognition (9-12). Retrospective epidemiological studies as well as studies in experimentally infected volunteers indicate that in the absence of antibodies, cellular immune responses can have a protective effect (9, 13, 14). Their role in cross-protection was demonstrated in a H1N1 infection study in non-human primates (NHP) (12). More recently the appreciation of the importance of non-neutralizing anti-influenza antibodies in conferring broad protection against variant strains, especially in the case of avian influenza viruses, has prompted research into their mechanism of action (via antibody dependent cellular cytotoxicity (ADCC), antibody dependent phagocytosis (ADP) (15) or complement activation (16-18) and vaccine strategies to induce these antibodies. New methods for faster vaccine production, the induction of T-cell responses and improvement of vaccine responses in the elderly have involved application of DNA, virus like particles (VLP), recombinant viral vectors and strategies to target vaccines to the appropriate antigen presenting cells (5, 19-23) and more recently the advent of mRNA-vaccines which facilitate fast responses as exemplified by COVID-19 vaccines from Pfizer and Moderna (24). Evaluation of the immunogenicity of these vaccines requires additional methods, besides the standard antibody ELISA, micro-neutralization and haemagglutination inhibition assays. Especially, proper assessment of adaptive cellular immune responses and function of the innate immune system in relation to non-neutralizing antibody effector function and induction of immune responses by these new vaccine modalities is needed (7).

Despite progress in the development of universal influenza vaccines, only few universal influenza vaccine candidates have progressed to clinical trials (25-27). In order to improve progress in the field of (universal) influenza vaccines, the National Institute of Allergy and Infectious Diseases (NIAID) has prepared a strategic plan outlining areas in our knowledge about influenza infection and immunity that require further investigation (7). Animal models have played an important role in preclinical evaluation of candidate influenza vaccines (28-30) and are still required during clinical development (7). While a number of species have been used, the most commonly used models to assess immunogenicity and efficacy against influenza virus infection are the mouse, ferret and NHP models. There are important differences between these species in immune function and susceptibility to influenza virus infection. Mice are not naturally susceptible to influenza and usually lethal infection models are used, that are based on extremely adapted human viruses. Ferrets are naturally susceptible to most human influenza viruses and recapitulate the natural course of infection. However, infection is often restricted to the upper airways and this model has the disadvantage that limited immunological tools are available, especially to study innate and cellular adaptive immune responses, which are especially important in the evaluation of "universal" vaccine strategies (31). NHP play an important role in influenza virus research and have been used to study pathogenesis as well as efficacy of preventive and therapeutic intervention strategies (32). Of the different animal models used in influenza virus research, NHP have a unique close homology to humans in most components of their immune system (33-35). For instance, similar T and B-cell subsets have been described in NHP (36). Moreover, the immunoglobulin gene germline repertoire is highly conserved between macaques and humans, which is important when induction of broadly neutralizing antibodies by new "universal" influenza vaccine strategies is studied (37, 38). In addition, structure and function of Fc receptors, which are

essential for the function of non-neutralizing antibodies, show many homologies between macaques and humans (39). Only very limited information is available on Fc receptors in ferrets and only reagents to detect the IgA receptor are available (31). Finally in NHP the innate immune system, including molecular pathways and antigen presenting cell subsets, are much more homologous to humans than what is seen in mice (34). NHP not only most closely reflect the human physiology, but also resemble humans in their clinical symptoms, limited pathology, pattern of viral replication, fever and cytokine and chemokine responses following influenza virus infection (40).
In conclusion, the strong immunological and physiological resemblances to humans make NHP a unique model in pre-clinical safety, immunogenicity and efficacy evaluation, particularly in relation to the new influenza virus vaccine delivery platforms being developed and for the evaluation of the important broadly neutralizing antibody, non-neutralizing antibody and cellular broadly protective immune responses. Evaluation in NHP is essential before the new "universal" influenza candidates can be evaluated in humans. Moreover, although challenge studies have been performed in humans (41), these are limited to the milder influenza strains and hampered by pre-existing immunity caused by previous exposures to influenza virus (42) limiting the value of the vaccine efficacy data that can be obtained.

Under project licence AVD[redacted]2016704 experiments were performed to refine the influenza virus infection model in macaques by evaluating aerosol delivery for infection with pandemic H1N1 (pH1N1) (43) influenza virus and highly pathogenic avian H5N1 influenza virus (doi.org/10.3390/v13020235). Experimental infection in NHP is typically performed by either intra-tracheal, or a combination of intra-tracheal, intra-nasal and intra-ocular virus inoculation. However, influenza virus infection in humans is assumed to be mainly caused by exposure to aerosols or droplets that enter the airways either via respiration, inhalation or via contact with contaminated surfaces (42). Our studies showed that aerosol delivery resulted in infection of the upper as well as lower respiratory tract for both pH1N1 and H5N1 influenza virus. However, infection with pH1N1 after aerosolized exposure resulted in lower levels of immune activation and inflammation than infection via combined intra-bronchial, intra-nasal and oral delivery. For H5N1 infection via aerosol exposure led to less severe disease than combined-route exposure. Hence, the route of exposure has clear consequence for disease pathogenesis. This allows for a fine tuning of the applied infection model in relation to the research question. When vaccine mediated protection against infection is studied then aerosol exposure would be the best option as it mimics best the situation in humans and allows adequate detection of reduction in virus replication. However, if protection against infection is difficult to achieve then the second objective should be protection against disease and in this case a combined-route exposure model should preferably be used. In conclusion aerosol delivery is now a well established infection model. However, it will still be needed to set up infection models for new influenza viruses that have not been used in NHP before.

The current project licence AVD[redacted]2016704 runs until 31-05-2022. However, the institute has recently been granted a project in which a novel mucosal influenza vaccine strategy will be evaluated, consisting of a systemic immunization with DNA followed by an oropharyngeal spray immunization with an adenovirus expressing the vaccine antigens. The hypothesis is that with this method strong local immune responses will be induced in the lungs. This prime/boost strategy involves in total a 83 week study period, which falls beyond the end date of the current project licence.

**3.2 Purpose**

3.2.1 Describe the project's immediate and ultimate goals. Describe to which extent achieving the project's immediate goal will contribute to achieving the ultimate goal.

- If applicable, describe all subobjectives

The goal of this project is to evaluate novel influenza virus vaccine candidates for occurrence of adverse effects, immunogenicity and capacity to protect against influenza virus infection in macaques. Both the capacity of new vaccine candidates to elicit a broad immune response, that not only protects against a homologous virus that is similar to the vaccine but also against heterologous viruses, as well as the immunogenicity of new influenza vaccine delivery platforms will be evaluated under this project application. The ultimate goal is to develop an influenza vaccine that can induce an immune response that is sufficiently broad to provide protection against seasonal influenza virus variants over a 5 year period (to obviate the need to vaccinate every year), is effective in elderly and can provide a degree of heterogeneous protection

that would lead to reduced morbidity and mortality caused by pandemic as well as highly pathogenic avian influenza viruses.
The main goal can be divided in 2 sub-goals:
1. Vaccine evaluation. Immunogenicity and efficacy to protect against infection will be evaluated using an appropriate influenza virus challenge strain in relation to the type of vaccine being used.
2. Set-up infection model for influenza viruses that have not yet been used in NHP at our institute and that are needed for vaccine evaluation.

3.2.2 Provide a justification for the project's feasibility.

At our institute we have been performing vaccine evaluation studies in NHP for over 20 years. Most vaccine candidates were directed against human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and tuberculosis. Since 2012 we have been working on influenza virus infection in macaques and the evaluation of vaccines against influenza (43-48). We have the appropriate facilities and experience to work with pathogenic viruses, including influenza virus, at DM-3 and ML-3 biosafety conditions. In addition, we have the appropriate immunological assays for assessment of cellular, humoral and innate immune responses against influenza. Our long-standing experience with pathogenic viruses, including influenza, and with vaccine evaluation guarantees that the animal studies describe in this proposal will be adequately performed.

3.2.3 Are, for conducting this project, other laws and regulations applicable that may affect the welfare of the animals and/or the feasibility of the project?

☒ No

☐ Yes > Describe which laws and regulations apply en describe the effects on the welfare of the animals and the feasibility of the project.

**3.3 Relevance**

3.3.1 What is the scientific and/or social relevance of the objectives described above?

Annual influenza virus epidemics cause considerable morbidity and mortality world-wide and especially affect more vulnerable groups like young children, the elderly and people with pulmonary diseases. In addition, there is the continuous threat of the emergence of new viral recombinants that can cause a pandemic. Previous pandemics, especially the 1918 pandemic, have caused millions of deaths. Finally, avian influenza viruses are widely spread and can occasionally infect humans. Mutations that lead to a strong increase in transmission have been described (49), indicating that also these viruses pose a continuous threat to the human population. Current influenza vaccine strategies and vaccine production methods are not adequate to deal with such emergencies. Even for protection against the current seasonal influenza viruses, annual vaccination of risk groups is necessary. Hence a vaccine that could offer protection against a broader range of viruses, including yet unknown recombinants and avian influenza would be of great benefit to the community. In addition, annual vaccination would no longer be necessary since a broadly protective vaccine would be effective over a period of at least five years against newly emerging variants. This has led the EU and the USA to invest in the development of so called "universal" influenza vaccines that would fulfil these criteria. Both the application of new delivery methods, for instance in the form of DNA, mRNA or viral vectors, as well as new vaccine modalities, such as mucosal delivery, require evaluation in appropriate animal models so that the level as well as the mechanism of protection can be adequately established, before these new vaccines can be tested in clinical studies.

3.3.2 Who are the project's stakeholders? Describe their specific interests.

The stakeholders for an influenza vaccine are the aforementioned target groups for whom protection from influenza infection and disease would increase their health and well-being. The vaccination of risk-groups and the resulting decrease in influenza burden would also be of great societal benefit. The animals involved in the experiments will not benefit and will experience moderate discomfort as a result of the experiments.

**3.4 Strategy**

3.4.1 Provide an overview of the overall design of the project (strategy). If applicable, describe the different phases in the project, the coherence, the milestones, selection points and decision criteria.

In order to evaluate that; a new influenza virus vaccine candidate is immunogenic, has the capacity to protect against infection and that no adverse effects occur, a vaccine evaluation experiment will be performed according to well established procedures, as described in Appendix 1. Typically, one or a number of immunizations are given over a certain period of time. After immunization the induction of T-cell and antibody immune responses is measured. The strength of these responses as well their breadth, i.e. the capacity to recognize not only homologous viruses that are similar to the vaccine but also heterologous viruses, is determined. Subsequently, the capacity of the vaccine to protect against infection is tested by experimental infection of the animals with influenza virus. The choice of the virus strain to be used for experimental infection will depend on the outcome of the evaluation of the immune responses and the stage of development of the vaccine. An entirely new vaccine concept may require that in first instance protection against infection by a homologous virus strain is tested. However, for most vaccine candidates protection against infection with a heterologous virus will be tested. Experimental infection will only be performed when the immunization has induced virus inhibiting antibody and cellular immune responses against the virus used for experimental infection so that protection against infection is possible. Whether protection is actually achieved depends on local interaction between cells of the immune system and local anti-viral antibodies with the virus and virus infected cells in the respiratory tract. This cannot be adequately modelled in an *in vitro* system and requires experimental infection of an animal. Also mucosal delivery methods and combinations of systemic as well as mucosal delivery can only be evaluated in a complex multi-organ environment. Ideally, the vaccine should provide a robust level of protection and be able to reduce disease and virus multiplication in animals that receive a standard virus dose via aerosol delivery. If protection against infection is unlikely and protection against disease or early immune inflammation needs to be established then combined exposure to the upper respiratory tract and lungs needs to be applied. A virus dose must be chosen that is not unrealistically high (above $10^7$ infectious particles), but high enough to lead to infection of all control animals.
In case proper evaluation of the capacity of a vaccine to protect against infection requires that a virus has to be used that has not been tested before in macaques at our institute then this virus will first be tested in a small number of animals. This to determine if all animals become infected and what the amount of virus multiplication is (Appendix 2). Either aerosol, intra-bronchial, oral, intranasal and intraocular inoculation is used, matching the method that will be used for the vaccine evaluation (Appendix 1).

3.4.2 Provide a justification for the strategy described above.

Vaccine candidates that fulfil the criteria for evaluation in NHP may be directly tested in a vaccine evaluation study (Appendix 1), if the influenza virus that will be used for establishing capacity of the vaccine to protect against infection has already been used in NHP at our institute. If this is not the case, the virus has to be tested first in an influenza virus infection study (Appendix 2). Also when efficacy against low dose aerosol infection has to be tested, a preceding influenza virus infection study (Appendix 2) is necessary.
*Vaccine evaluation in macaques.*
For this type of experiment animals will be immunized either once or they will receive a number of immunizations over a certain period of time. During the study animals will be monitored for adverse effects of the vaccine, including monitoring of general behaviour and health. Blood and occasionally nasal washes and lung lavages will be taken to measure induction of systemic as well as local immune responses. When adequate immune responses are induced that indicate that protection against infection might be achieved, the efficacy against infection will be tested by experimental infection with influenza virus. A group of non-vaccinated animals will be included as infection controls.
Vaccines that will be tested in NHP have to be in the final stages of preclinical development and require this last validation step in order to assure that there are no adverse effects that were missed in the preliminary studies in other species and that they are effective in an animal species that has an immune system closely related to humans. Additional criteria for vaccine evaluation are: a) the vaccine strategy must be novel, for instance with regards to choice of antigen, formulation, route of application, that have not been tested before in similar NHP studies, b) demonstration that the vaccine or vaccine components are non-toxic, c) when specific host molecules are targeted then cross recognition of macaque homologues must have been demonstrated, d) the vaccine cannot be adequately tested in other than NHP animal models, for instance due to the mechanism of action or the type of immunological assessment needed, e) preferably immunogenicity of vaccine candidates should have been proven in other species, unless this is not possible because the specific vaccine modality used does not work in other species.

*Influenza virus infection in macaques*.
In order to establish infectivity and pathogenicity of a new virus that has not been tested previously in NHP at our institute, a small number of animals will be infected and monitored for clinical symptoms, fever, body weight and changes in blood parameters. Development of lesions in the lungs will be monitored by (PET)-CT analysis. Nasal and tracheal swabs will be taken to determine if the animals have become infected and determine the magnitude of virus multiplication. Proper application in vaccine evaluation requires that in these infection studies > 80% of the animals become infected and that the amount of virus produced in the trachea over the infection period is clearly measurable and that the variation between the animals is sufficiently low to allow measurement of reduction in virus load in vaccinated animals with less than 10 animals per group. In case these parameters are not achieved then the experiment will be repeated with a 10-100 times higher dose. In case any of the animals reaches the humane endpoint within the first four days after infection then a 10-100 times lower virus dose will be evaluated. The same criteria will be used to determine whether the aerosol infection model is sufficiently robust.

3.4.3 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

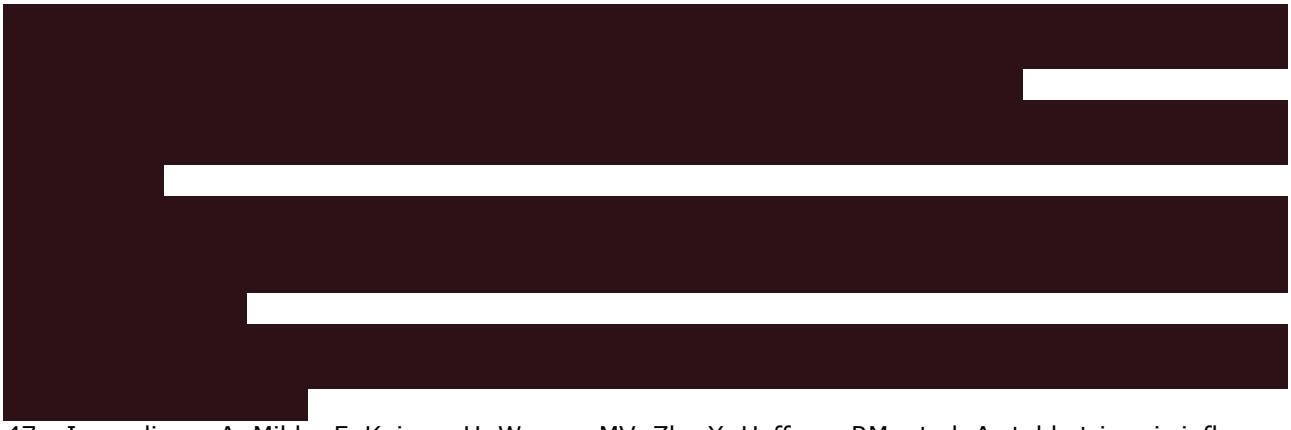
| Serial number | Type of animal procedure |
|---|---|
| 1 | Influenza vaccine evaluation in macaques |
| 2 | Establishment of a new influenza infection model in macaques |
| 3 | |
| 4 | |
| 5 | |
| 6 | |
| 7 | |
| 8 | |
| 9 | |
| 10 | |

**Referenties**


1. WHO. Influenza (Seasonal) [Available from: https://www.who.int/en/news-room/fact-sheets/detail/influenza-(seasonal).
2. EC. Influenza [Available from: https://ec.europa.eu/health/vaccination/influenza_en.
3. de Vries RD, Altenburg AF, Rimmelzwaan GF. Universal influenza vaccines, science fiction or soon reality? Expert Rev Vaccines. 2015:1-3.
4. Osterhaus A, Fouchier R, Rimmelzwaan G. Towards universal influenza vaccines? Philos Trans R Soc Lond B Biol Sci. 2011;366:2766-73.
5. Krammer F, Palese P. Advances in the development of influenza virus vaccines. Nat Rev Drug Discov. 2015;14:167-82.
6. Valenciano M, Kissling E, Reuss A, Rizzo C, Gherasim A, Horvath JK, et al. Vaccine effectiveness in preventing laboratory-confirmed influenza in primary care patients in a season of co-circulation of influenza A(H1N1)pdm09, B and drifted A(H3N2), I-MOVE Multicentre Case-Control Study, Europe 2014/15. Euro Surveill. 2016;21(7):pii=30139.
7. Erbelding EJ, Post D, Stemmy E, Roberts PC, Augustine AD, Ferguson S, et al. A Universal Influenza Vaccine: The Strategic Plan for the National Institute of Allergy and Infectious Diseases. J Infect Dis. 2018.
8. Krammer F, Fouchier RAM, Eichelberger MC, Webby RJ, Shaw-Saliba K, Wan H, et al. NAction! How Can Neuraminidase-Based Immunity Contribute to Better Influenza Virus Vaccines? MBio. 2018;9(2).
9. McMichael AJ, Gotch FM, Noble GR, Beare PA. Cytotoxic T-cell immunity to influenza. New England Journal of Medicine. 1983;309:13-7.
10. Sridhar S, Begom S, Bermingham A, Hoschler K, Adamson W, Carman W, et al. Cellular immune correlates of protection against symptomatic pandemic influenza. Nat Med. 2013:10.

11. Wilkinson TM, Li CK, Chui CS, Huang AK, Perkins M, Liebner JC, et al. Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans. Nat Med. 2012;18:274-80.
12. Weinfurter JT, Brunner K, Capuano SV, III, Li C, Broman KW, Kawaoka Y, et al. Cross-reactive T cells are involved in rapid clearance of 2009 pandemic H1N1 influenza virus in nonhuman primates. PLoS Pathog. 2011;7:e1002381.
13. Epstein SL. Prior H1N1 influenza infection and susceptibility of Cleveland Family Study participants during the H2N2 pandemic of 1957: an experiment of nature. J Infect Dis. 2006;193:49-53.
14. McElhaney JE, Xie D, Hager WD, Barry MB, Wang Y, Kleppinger A, et al. T cell responses are better correlates of vaccine protection in the elderly. J Immunol. 2006;176:6333-9.
[redacted]
16. Jegaskanda S, Amarasena TH, Laurie KL, Tan HX, Butler J, Parsons MS, et al. Standard trivalent influenza virus protein vaccination does not prime antibody-dependent cellular cytotoxicity in macaques. J Virol. 2013;87:13706-18.
17. Jegaskanda S, Reading PC, Kent SJ. Influenza-specific antibody-dependent cellular cytotoxicity: toward a universal influenza vaccine. J Immunol. 2014;193:469-75.
18. Henry Dunand CJ, Leon PE, Huang M, Choi A, Chromikova V, Ho IY, et al. Both Neutralizing and Non-Neutralizing Human H7N9 Influenza Vaccine-Induced Monoclonal Antibodies Confer Protection. Cell Host & Microbe. 2016;19:800-13.
19. Florek NW, Weinfurter JT, Jegaskanda S, Brewoo JN, Powell TD, Young GR, et al. Modified vaccinia virus Ankara encoding influenza virus hemagglutinin induces heterosubtypic immunity in macaques. J Virol. 2014;88(22):13418-28.
20. Deliyannis G, Boyle JS, Brady JL, Brown LE, Lew AM. A fusion DNA vaccine that targets antigen-presenting cells increases protection from viral challenge. Proc Natl Acad Sci U S A. 2000;97(12):6676-80.
21. Fossum E, Grodeland G, Terhorst D, Tveita AA, Vikse E, Mjaaland S, et al. Vaccine molecules targeting Xcr1 on cross-presenting DCs induce protective CD8+ T-cell responses against influenza virus. Eur J Immunol. 2015;45:624-35.
22. Grodeland G, Mjaaland S, Roux KH, Fredriksen AB, Bogen B. DNA vaccine that targets hemagglutinin to MHC class II molecules rapidly induces antibody-mediated protection against influenza. J Immunol. 2013;191:3221-31.
23. Laddy DJ, Yan J, Khan AS, Andersen H, Cohn A, Greenhouse J, et al. Electroporation of synthetic DNA antigens offers protection in nonhuman primates challenged with highly pathogenic avian influenza virus. J Virol. 2009;83(9):4624-30.
24. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov. 2018;17(4):261-79.
25. Nachbagauer R, Feser J, Naficy A, Bernstein DI, Guptill J, Walter EB, et al. A chimeric hemagglutinin-based universal influenza virus vaccine approach induces broad and long-lasting immunity in a randomized, placebo-controlled phase I trial. Nature Medicine. 2020.
26. Atsmon J, Kate-Ilovitz E, Shaikevich D, Singer Y, Volokhov I, Haim KY, et al. Safety and immunogenicity of multimeric-001--a novel universal influenza vaccine. J Clin Immunol. 2012;32:595-603.
27. van Doorn E, Liu H, Ben-Yedidia T, Hassin S, Visontai I, Norley S, et al. Evaluating the immunogenicity and safety of a BiondVax-developed universal influenza vaccine (Multimeric-001) either as a standalone vaccine or as a primer to H5N1 influenza vaccine: Phase IIb study protocol. Medicine. 2017;96:e6339.
28. Bodewes R, Rimmelzwaan GF, Osterhaus AD. Animal models for the preclinical evaluation of candidate influenza vaccines. Expert Rev Vaccines. 2010;9(1):59-72.
29. Bouvier NM, Lowen AC. Animal Models for Influenza Virus Pathogenesis and Transmission. Viruses. 2010;2(8):1530-63.
[redacted]
31. Albrecht RA, Liu WC, Sant AJ, Tompkins SM, Pekosz A, Meliopoulos V, et al. Moving Forward: Recent Developments for the Ferret Biomedical Research Model. mBio. 2018;9(4).
32. Davis AS, Taubenberger JK, Bray M. The use of nonhuman primates in research on seasonal, pandemic and avian influenza, 1893-2014. Antiviral Res. 2015.

33. Sanghavi SK, Shankarappa R, Reinhart TA. Genetic analysis of Toll/Interleukin-1 Receptor (TIR) domain sequences from rhesus macaque Toll-like receptors (TLRs) 1-10 reveals high homology to human TLR/TIR sequences. Immunogenetics. 2004;56(9):667-74.
34. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. J Immunol. 2004;172(5):2731-8.
35. Jacobsen FW, Padaki R, Morris AE, Aldrich TL, Armitage RJ, Allen MJ, et al. Molecular and functional characterization of cynomolgus monkey IgG subclasses. J Immunol. 2011;186:341-9.
36. Demberg T, Robert-Guroff M. B-Cells and the Use of Non-Human Primates for Evaluation of HIV Vaccine Candidates. Curr HIV Res. 2015;13(6):462-78.
37. Link JM, Hellinger MA, Schroeder HW, Jr. The Rhesus monkey immunoglobulin IGHD and IGHJ germline repertoire. Immunogenetics. 2002;54:240-50.
38. Sundling C, Li Y, Huynh N, Poulsen C, Wilson R, O'Dell S, et al. High-resolution definition of vaccine-elicited B cell responses against the HIV primary receptor binding site. Sci Transl Med. 2012;4(142):142ra96.
39. Hogarth PM, Anania JC, Wines BD. The FcgammaR of humans and non-human primates and their interaction with IgG: implications for induction of inflammation, resistance to infection and the use of therapeutic monoclonal antibodies. CurrTopMicrobiolImmunol. 2014;382:321-52.
40. O'Donnell CD, Subbarao K. The contribution of animal models to the understanding of the host range and virulence of influenza A viruses. MicrobesInfect. 2011;13:502-15.
41. Carrat F, Vergu E, Ferguson NM, Lemaitre M, Cauchemez S, Leach S, et al. Time lines of infection and disease in human influenza: a review of volunteer challenge studies. Am J Epidemiol. 2008;167(7):775-85.
42. Killingley B, Enstone J, Booy R, Hayward A, Oxford J, Ferguson N, et al. Potential role of human challenge studies for investigation of influenza transmission. Lancet Infect Dis. 2011;11:879-86.



47. Impagliazzo A, Milder F, Kuipers H, Wagner MV, Zhu X, Hoffman RM, et al. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. Science. 2015;349:1301-6.

49. Linster M, van Boheemen S, de Graaf M, Schrauwen EJ, Lexmond P, Manz B, et al. Identification, characterization, and natural selection of mutations driving airborne transmission of A/H5N1 virus. Cell. 2014;157:329-39.