

Viral vector-based influenza vaccines

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ABSTRACT

Antigenic drift of seasonal influenza viruses and the occasional introduction of influenza viruses of novel subtypes into the human population complicate the timely production of effective vaccines that antigenically match the virus strains that cause epidemic or pandemic outbreaks. The development of game-changing vaccines that induce broadly protective immunity against a wide variety of influenza viruses is an unmet need, in which recombinant viral vectors may provide. Use of viral vectors allows the delivery of any influenza virus antigen, or derivative thereof, to the immune system, resulting in the optimal induction of virus-specific B- and T-cell responses against this antigen of choice. This systematic review discusses results obtained with vectored influenza virus vaccines and advantages and disadvantages of the currently available viral vectors.

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Introduction

Influenza viruses belong to the family of *Orthomyxoviridae*, are an important cause of acute respiratory infections and cause annual epidemics in the human population. Although in most cases infections are self-limiting and restricted to the upper respiratory tract, certain patient groups (such as the elderly) are at risk of developing complications leading to high morbidity and mortality. Vaccines against circulating influenza strains are readily available and are trivalent or quadrivalent, designed to protect against influenza viruses of both the A(H1N1) and A(H3N2) subtype, and against one or both lineages of influenza B virus.

Several different vaccine formulations are available: trivalent or quadrivalent inactivated virus vaccines (TIV or QIV, either whole virus, split virus or subunit vaccines) or live attenuated influenza virus vaccines (LAIV). Most vaccines are produced in embryonated chicken eggs, but vaccines produced in mammalian or insect cells are also available. Inactivated vaccines are administered intramuscularly (IM) or sometimes intradermally and predominantly aim at the induction of serum antibody responses against the viral hemagglutinin (HA) and neuraminidase (NA) to a lesser extent. Protection from disease is mainly mediated by virus neutralizing antibodies against HA, but NA-specific antibodies also contribute to protective immunity.¹ Currently licensed LAIV are administered locally via nasal spray. Viruses are attenuated by the choice of a viral backbone of cold-adapted viruses and are therefore temperature-sensitive and replicate only locally after administration at the mucosa of the nasopharynx.² In addition to serum antibodies, immunization with LAIV also induces mucosal antibodies and cytotoxic T-lymphocytes (CTL).

Although currently available influenza vaccines are effective in reducing morbidity and mortality caused by seasonal influenza viruses, they have several limitations. Mainly, continuous antigenic

drift of seasonal influenza viruses complicates the production of effective vaccines. The vaccine strains need to be updated almost annually in order to achieve a good antigenic match with the epidemic virus strains. If the vaccine strains do not antigenically match the circulating strains, vaccine efficacy is reduced considerably, as was the case in the 2014–2015 influenza season.^{3–5} Furthermore, the seasonal influenza vaccines will afford little or no protection against antigenically distinct pandemic influenza viruses, which are often of alternative subtypes to which antibodies are virtually absent in the human population. During the last decades zoonotic transmissions of highly pathogenic avian influenza viruses, in particular those of the H5N1 subtype, have been reported regularly. The capacity of A(H5N1) and avian viruses of other subtypes including A(H5N6),⁶ A(H7N7),⁷ A(H7N9),⁸ A(H9N2)⁹ and A(H10N8)¹⁰ to infect humans fuelled the fear for a pandemic outbreak caused by any of these viruses.

H5N1 vaccines that were produced according the procedures used for the production of seasonal influenza vaccines proved to be poorly immunogenic and in most cases the use of adjuvants was required for the efficient induction of protective antibody levels.¹¹ Furthermore, the pandemic of 2009 caused by swine-origin influenza viruses of the A(H1N1) subtype (H1N1pdm09) taught an important lesson. The production of tailor made pandemic influenza vaccine proved to be a time-consuming process and in many countries vaccines became available after the peak of the pandemic.¹²

These limitations of the currently available vaccine production technologies and vaccines underscore the pressing need for game-changing vaccines. In addition to improving immunogenicity in the high risk groups, novel vaccines are required that induce long-lasting immunity against a wide range of influenza viruses and that can be produced rapidly in the face of a pandemic outbreak. To

improve immunogenicity of influenza vaccines specifically in the elderly, high-dose vaccines and an adjuvanted vaccine have been developed. The latter has been in use in Europe and the US since 1997 and 2015, respectively.

The use of viral vectors for influenza vaccine production may be a solution to some of the problems discussed above. In this review we discuss various viral vectors that have been tested as candidate influenza vaccines in animal models and in clinical trials. Most viral vectors are considered live vaccines but their replication is attenuated or even deficient. Therefore, vector-based vaccines are considered safe in general and some of them can even be safely used in immunocompromised. Despite their attenuated phenotype, viral vectors are immunogenic and induce virus-specific antibody and T cell responses after systemic or parenteral administration. Additionally, most viral vectors can easily be propagated to high virus titers and it is relatively easy to insert genes encoding antigens of choice into the vector. Viral vector technology also allows the production of modified influenza viral antigens *in vivo*. These modifications can improve the immunogenicity of the influenza viral proteins or alter the specificity of the immune response. In this review, we discuss reports on vectored influenza vaccines and discuss their advantages and disadvantages.

Vectored influenza vaccines

Pox virus vectors

Smallpox, caused by variola virus, was the first viral disease that was widely prevented and eradicated by vaccination. Originally, Edward Jenner was able to prevent experimental smallpox infection of humans by priming the immune system with the closely related cowpox virus. Vaccinia virus (VV), closely related to the causative agent of cowpox, was thereafter used as one of the vaccines to eradicate smallpox. Since VV has optimal properties to be used as a viral vector, soon after its initial use as cloning vector in 1982^{13,14} VV was used as a vaccine vector to express influenza virus antigens. Smith *et al* were the first to generate VV expressing the influenza HA gene and this vaccine was able to induce an antibody response in rabbits and could protect hamsters from lethal challenge.¹⁵ Since then, recombinant VV were designed that express all influenza virus proteins.¹⁶ Although VV vectors expressing influenza antigens were capable of inducing protective immune responses in various animal models, substantial reactogenicity of this vector was frequently observed, which has been addressed by the use of further attenuated and/or replication-deficient strains of VV. An overview of poxvirus-based influenza vaccines can be found in Table 1.

Modified vaccinia virus Ankara (MVA) vectors

MVA, an attenuated VV strain, is derived from chorioallantoic vaccinia virus Ankara through serial passaging in chicken embryo fibroblasts,^{17,18} resulting in major deletions in the viral genome that influenced many virulence and immune evasion factors.¹⁹⁻²¹ Consequently, MVA replication is highly restricted to avian cells and MVA is unable to produce infectious progeny in most mammalian cell types.²² Since MVA is replication-deficient in mammalian cells and therefore lowly reactogenic in

humans, it is an attractive vector for vaccination purposes. This was demonstrated in field trials, where MVA was successfully used as a safe smallpox vaccine in over 120,000 individuals in the absence of any serious adverse events.²³

However, the use of MVA as a vaccine vector has multiple alternative advantages. Notably, MVA safety was confirmed in various *in vivo* models, including avian species and mammals with immunodeficiencies,²⁴⁻²⁷ leading to classification of MVA as a biosafety level 1 (BSL-1) pathogen. Additional advantages of MVA as a vaccine vector include: easy insertion of antigens of interest into the viral genome, transient expression of heterologous antigens *in vivo* and induction of both humoral and cellular responses in animal models and humans. Finally, an interesting characteristic of MVA is that compared to VV, MVA has lost the capacity to evade the host innate immune system.²⁸⁻³⁵ Consequently, vaccination with MVA has an intrinsic immunostimulatory activity (potentially comparable to adjuvants used in combination with vaccination) that leads to rapid influx of various types of immune cells.³⁴ Although a potential negative effect of pre-existing vector immunity on immunogenicity is always a concern with the use of vectored vaccines, this does not seem to be a major problem with MVA-based vaccines. It has been shown in humans that a second booster vaccination with a MVA expressing an influenza virus HA, still resulted in potent antibody responses against the protein of interest. Similar observations were made in other studies with MVA expressing other proteins (reviewed in ³⁶). This indicates that recombinant MVA remain immunogenic, despite vector immunity.











MVA holds great promise as a vaccine vector and was initially shown to be a promising influenza vaccine in 1994 by Sutter *et al*.³⁷ This vaccine was engineered to express the HA and nucleoprotein (NP) gene from influenza virus A/PR/8/34. In addition, recombinant MVA expressing other proteins from various influenza strains were generated and tested in animal models.

MVA-HA vaccines

To induce sterile immunity against influenza viruses, HA is the surface antigen of choice since it efficiently stimulates B-cell responses and the production of virus neutralizing antibodies (VN) *in vivo*. Therefore, MVA vector vaccines expressing HA of various subtypes have been constructed and tested in animal models. It should be noted however that most antibodies directed to HA are strain-specific and display poor cross-reactivity with HAs of alternative subtypes, or even with HA molecules from other viruses of the same subtype. Therefore, MVA-HA vaccines often offer protection from infection with the homologous influenza A virus, but not or poorly against infection with heterologous viruses.




Recombinant MVAs expressing the HA gene of the H1N1pdm09 influenza virus A/California/07/09 have been tested for immunogenicity in mice, ferrets and macaques. In different studies, mice could be fully protected from disease after challenge infection with the homologous virus and protection correlated with the induction of VN antibody and T-cell responses.^{38,39} In addition, intra-subtypic cross-immunity was observed to some extent as MVA-H1(A/Cal/07/09) could also protect mice from infection with various A(H1N1) swine influenza viruses.³⁸ Immunization of ferrets with a similar recombinant MVA induced robust antibody responses and partially

Table 1. Overview of poxvirus-based influenza vaccines.

Vector	Model	Antigen	Modification	Subtype	Reference
Poxviruses		HA	n/a	H1N1, H5N1	37,38,43-45,68
		HA	HAstem	H5N1	51
		HA	Mosaic	H5	63
		NP	n/a	H5N1	51
		M1	n/a	H5N1	51
		M2	n/a	H5N1	51
		PB2	n/a	H5N1	51
		NA	n/a	H5N1	38,67
		HA/NP	n/a, HAstem	H1N1, H5N1	36,51-53,68
		NP/M1	n/a	H3N2	56,57
		HA/NA	n/a	H5N1	54,68
		HA/M2	HAstem, M2e repeats	H1N1, H5N1, H7N2, H9N2	51
		HA/NP/M2	HAstem, M2e repeats	H1N1, H5N1, H7N2, H9N2	51
		HA/NP/NA/M1/M2	n/a	H5N1	54
				HA	n/a
		HA NP/M1	n/a n/a	H5N1 H3N2	66 57
		HA	n/a	H1N1, H5, H5N1, H5N8, H7N2, H7N7, H7N9, H9N2	26,77-81,83,84,89-93
NP		n/a	?		93
HA/NP		n/a	H5		93
HA/NA		n/a	H5N1		94,95
		NP/M1	n/a	H3N2	55,57
		HA	n/a	H5, H5N1	81,85,86,88
		HA	n/a	?	77
		HA	n/a	H3N8	96
		HA	n/a	H3N8 H3N8	49,69-74
		NP	n/a		49
		HA	n/a	H2N2	15
		HA	n/a	H2N2	15

(Continued)

Table 1. (Continued)

Vector	Model	Antigen	Modification	Subtype	Reference
		HA	n/a	H5N1, H5N8	79,96
		HA	n/a	H1N1, H5N1	40,46
		NP	n/a	H5N1	40
		HA/NP	n/a	H1N1, H5N1	40
		HA	n/a	H5N1	47,48
		NP/M1	n/a	H3N2	58-62

protected from challenge infection with an antigenically closely related H1N1pdm09 virus, A/NL/602/09.⁴⁰ Macaques were also fully protected from H1N1pdm09 virus infection (A/Norway/3487/09) by 2 immunizations with a MVA-based H1N1pdm09 vaccine.⁴¹ Taken together, these data indicate that an MVA-based vaccine is able to induce protective immunity against the virus that caused the influenza pandemic in 2009, but the extent of cross-protection against unrelated H1N1 viruses is limited.

Highly pathogenic avian influenza viruses of the H5N1 subtype cause endemic outbreaks in poultry. However, since 1997, zoonotic transmission of various A(H5) viruses have caused many cases of human infection^{6,42} of which almost 400 had a fatal outcome. The zoonotic transmission of A(H5N1) viruses, their continuous circulation in wild and domestic birds and the fact that only a few amino acid substitutions are necessary to confer transmissibility between mammals,⁴³ underscore the pandemic potential of these viruses. The circulation of A(H5N1) viruses belonging to antigenically distinct clades has complicated vaccine development and has necessitated the selection of various prototypic vaccine strains. Recombinant MVA vaccines expressing HA genes derived from various A(H5N1) strains have been constructed and tested for protective efficacy against viruses belonging to different clades in various animal models. Vaccination with MVA expressing the HA gene of influenza virus A/Vietnam/1194/04 (clade 1) completely protected mice and chickens from infection with the homologous virus and also offered mice protection against infection with influenza viruses A/HK/156/97 (clade 0) and A/Indonesia/5/05 (clade 2.1).^{26,44,45} With regard to inducing antibodies that cross-react with the HA of viruses belonging to heterologous clades of A(H5N1) viruses, MVA-H5(A/Vietnam/04) performed superior compared to MVA expressing HA of influenza A viruses A/Indonesia/5/05 (clade 2.1), A/HK/156/97 (clade 0), A/Turkey/Turkey/1/05 (clade 2.2), A/Chicken/Egypt/3/06 (clade 2.2) or A/Anhui/1/05 (clade 2.3).^{45,46} Finally, immunization with MVA-H5(A/Vietnam/04) protected macaques from challenge infection with the both homologous virus and clade 2.1 virus A/Indonesia/5/05.⁴⁷ This favorable outcome justified testing of MVA-H5(A/Vietnam/04) in a phase 1/2a clinical trial that showed that this vaccine candidate was immunogenic in man. VN antibodies were induced against the homologous strain that cross-reacted not only with heterologous A(H5N1) viruses, but even with a newly emerging A(H5N8) avian influenza virus.^{48,49} The vaccine was well tolerated and serious adverse events

were not observed. Collectively, these data show that MVA-based A(H5) vaccines are safe and able to induce cross-clade specific antibodies at levels that are considered protective.

In addition, MVAs expressing HA genes derived from A(H3) and A(H7) influenza viruses have been tested in animal models. A MVA vaccine expressing the HA gene of an A(H3N8) equine influenza virus (A/equine/Kentucky/1/81) was tested in ponies and was shown to induce antibody and T-cell responses, and afforded protection from disease caused by challenge infection with the homologous virus.⁵⁰ Recently, avian influenza viruses of the A(H7N9) subtype have caused frequent infection of humans, especially in Southeast China, often with fatal outcome. A candidate MVA-based vaccine expressing the HA gene of one of these viruses (A/Shanghai/2/13) was constructed and tested in ferrets. It was shown that immunization with MVA-H7(A/Shanghai/2/13) induced VN antibodies and offered partial protection against challenge infection with a closely-related A(H7N9) virus.⁵¹

MVA-NP, MVA-M1, MVA-NA and MVA-PB1 vaccines

Whereas HA is regarded as the antigen of choice for the induction of VN antibody responses, the more conserved internal NP and Matrix 1 (M1) protein are often regarded as antigens of choice for the induction of T-cell responses, in particular CD8⁺ CTL responses. In general, the HA-based vaccines induce a relatively narrow antibody response, which afford little cross-protective immunity to viruses of other subtypes. In contrast, the use of more conserved influenza virus proteins in vaccines may lead to the induction of T-cells directed to epitopes that are shared by influenza viruses of various subtypes and may confer broader protection. For the induction of broadly-protective immunity, MVA-M1, MVA-NA, MVA-PB1 and MVA-NP vaccines were constructed and tested in animal models. Of note, vaccines that aim at the induction of T cell immunity exclusively will not afford sterile immunity, because initial infection and some degree of virus replication cannot be prevented. However, CTL recognize and eliminate virus-infected cells and thereby contribute to viral clearance and recovery.

Hessel *et al* constructed a recombinant MVA expressing the NP gene of an A(H5N1) virus (MVA-NP(A/Vietnam/1203/04)). Immunization of mice with this vaccine candidate not only protected animals from infection with the homologous virus, but also against infection with viruses of the A(H7N1) and A(H9N2) subtype.⁵² However, a similar MVA-NP(A/Vietnam/1203/04)

construct failed to induce protective immunity in macaques.⁴¹ The use of MVA expressing the M1 or PB1 gene from an A(H5N1) virus also failed to induce protective immunity against infection.⁵² MVA expressing the NP gene of an equine A(H3N8) virus (MVA-NP(A/Equine/Kentucky/1/81)) offered ponies partial protection from infection, but only after initial priming with a DNA vaccine expressing the same antigen.⁵⁰ MVA expressing the NA gene from an H1N1pdm09 virus afforded partial protection against H1N1pdm09 challenge infection.³⁹

Simultaneous delivery of multiple influenza virus antigens by MVA

In order to elicit both protective antibody and T-cell responses simultaneously, MVA expressing both the HA and NP gene have been constructed. In mouse studies, the use of a MVA-H1+NP(A/PR/8/34) vaccine induced virus-specific antibodies and T-cell responses and fully protected mice from infection with the homologous virus and partially protected against infection with a unrelated A(H3N2) virus.^{37,53} Similarly, an MVA-H1+NP was constructed that contained the HA gene of an H1N1pdm09 and the NP gene of a A(H5N1, A/Vietnam/1203/04) virus. In mice, vaccination afforded complete protection from infection with the homologous A(H1N1) and A(H5N1) strains and partial protection from infection with a virus of the A(H3N2) subtype.⁵⁴ A similar MVA-H1+NP construct was tested in macaques, full protection was observed against infection with H1N1pdm09 virus. When the HA gene was replaced by that of an A(H5N1) virus, macaques were only partially protected from infection with the H1N1pdm09 virus.⁴¹ An MVA simultaneously expressing the HA and NA genes of A(H5N1) virus A/Vietnam/1203/04 and the IL-15 gene was tested in mice and was shown to afford protection against infection with the A(H5N1) virus.⁵⁵

A MVA vaccine designed to induce T-cell responses, expressing the NP and M1 genes of an A(H3N2) virus was extensively tested; first in animal models and then in clinical trials. Immunization of mice with MVA-NP+M1(A/Panama/2007/09) afforded protection against development of severe disease after infection with H1N1pdm09 and A(H3N2) influenza viruses, but not against infection with the mouse-adapted A(H1N1) virus A/PR/8/34. In these studies the recombinant MVA was given in adjunct with recombinant adenovirus expressing the same genes. With the same vaccination strategy chickens were protected from infection with a A(H7N7) virus. Thus with recombinant MVA expressing the conserved NP and M1 genes simultaneously broad immune responses are induced that protect against various subtypes of influenza A virus.⁵⁶⁻⁵⁹ This vaccination regimen proved immunogenic in pigs, but the protective potential was not tested in this species.⁵⁸ An MVA-NP+M1 vaccine was subsequently tested in phase 1/2a clinical trials and was shown to induce virus-specific CD8⁺ T-cells in humans and protect from experimental challenge infection with an A(H3N2) virus.^{58,60,61} Furthermore, this vaccination regimen was again shown to be safe and immunogenic in the elderly,⁶² and could be co-administered with TIV.⁶³

Universal influenza vaccines on basis of MVA

Because of the variable nature of influenza viruses and the extensive antigenic variation they display, the availability of so-called universal vaccines is highly desirable. Some of the MVAs

that were discussed above induced cross-reactive immune responses, in particular based on the induction of virus-specific T cells. Commonly unmodified antigens are being used, but currently various modifications of viral proteins are being tested in order to design vaccines that induce broader immunity. Some of these modified influenza viral antigens are now being expressed by recombinant viral vectors, including MVA, and are being tested in animal models.

Recently, Kamlangdee *et al* generated an MVA-H5Mosaic construct, an MVA expressing a computationally generated mosaic A(H5) gene reflecting gene sequences of 2,145 A(H5N1) virus isolates. The MVA-H5Mosaic vaccine was capable of inducing antibodies that reacted with A(H5N1) viruses from all clades, but that did not cross-react with viruses of other HA subtypes. Interestingly, immunized mice were not only protected from infection with various A(H5N1) viruses, but also from infection with A/PR/8/34, a virus of the A(H1N1) subtype. Protection from challenge infection with an A(H3N2) could not be achieved.⁶⁴

Another vaccine approach of interest is the design of vaccines that can induce broadly-reactive and VN antibodies to the conserved stalk region of the HA molecule.⁶⁵ A single study has performed vaccination challenge experiments with MVA expressing the HA-stalk, either alone or in combination with other antigens.⁵² The use of MVA-H5Stalk(A/Vietnam/1203/04) alone or in combination with an MVA expressing 4 M2e repeats from A(H5N1), A(H9N2), A(H7N2) and A(H1N1) did not afford protective immunity. However, when the MVA-H5Stalk (with or without the MVA expressing M2e) construct was combined with an MVA expressing an NP gene (MVA-NP(A/Vietnam/1203/04)), mice were protected from infection with A(H5N1), A(H7N1) and A(H9N2) viruses.⁵² Co-administration of an NP expressing vaccine seemed essential in achieving cross-protection in these studies, corresponding to the induction of broadly-reactive virus-specific CD4⁺ and CD8⁺ T-cells.

Thus, it was shown that influenza virus antigens can be modified to optimize induction of (cross-reactive) immune responses and these modified viral antigens can readily be expressed by recombinant MVA vectors. Thus MVA provides an ideal platform for the expression of modified influenza viral proteins, smartly designed for the induction of broadly protective immunity.

NYVAC, Raccoonpox, Canarypox and Fowlpox vectors

Next to MVA, other attenuated poxviruses have been used as viral vectors for the development of candidate influenza vaccines. Immunization of chickens with recombinant NYVAC, a VV strain highly attenuated by deletion of 18 open reading frames from the viral genome,⁶⁶ expressing the HA gene of an avian A(H5N1) virus (NYVAC-H5(A/Chicken/Indonesia/7/04)), afforded protection against infection with a heterologous A(H5N2) virus.⁶⁷ Recombinant Raccoonpox (RCN) viruses expressing the HA, NA or NP genes of A(H5N1) influenza virus A/Vietnam/1203/04 were constructed and evaluated for their protective capacity in mice. Interestingly, protection from A(H5N1) challenge infection could be demonstrated but was dependent on the route of administration. Intra-dermal immunization with RCN-HA afforded protection, protection was also observed with RCN-NA but only after intra-nasal administration.^{68,69}

Canarypox (CNPV) is a host-restricted member of the poxvirus family that is unable to produce infectious progeny virus in mammalian cells, making it potentially safe as a viral vector for human use. At present, recombinant CNPV are used as influenza vector vaccines for vaccination of horses against equine influenza of the A (H3N8) subtype. Recombinant CNPV vaccines expressing HA genes of various A(H3N8) strains were immunogenic in horses and protected against infection with virus of this subtype.⁷⁰⁻⁷⁵ Furthermore, maternal antibodies induced by CNPV-HA were passively transferred to foals of vaccinated pregnant horses.⁷⁶ In addition, CNPV was also used as a vaccine vector to protect pigs and cats from avian influenza virus of the A(H5N1) subtype. Pigs were vaccinated with CNPV-HA(A/Chicken/Indonesia/7/03) and proved to be protected from infection with an unrelated virus of the A(H5N2) subtype.⁶⁷ In cats, the same vaccine afforded protection from infection with various A(H5N1) viruses.⁷⁷

Fowlpox virus (FPV) is the causative agent of an economically important disease of chickens. However, several attenuated FPV vector vaccines expressing influenza virus antigens are currently licensed as A(H5N1) influenza vaccines for use in poultry. Initial studies performed in 1998 showed that FPV-HA vaccines could protect chickens and turkeys from experimental challenge infections.⁷⁸ Comparable to MVA, it was recently shown that FPV as a vector efficiently induces both B- and T-cell responses in chickens,⁷⁹ however it has also been shown that pre-existing immunity to FPV or influenza antigens could pose a potential problem when using FPV-based influenza vaccines.⁸⁰

An experimental FPV-based A(H5) vaccine, expressing the HA gene of an A(H5N8) virus (A/Turkey/Ireland/1378/83) was extensively tested in chickens and was shown to offer protection against infection with the homologous virus, but also against viruses of the A(H5N1), A(H5N2), A(H5N3), A(H5N8) and A(H5N9) subtypes.⁸¹⁻⁸³ Various other FPV-H5 vaccines were constructed and tested in chickens and ducks and proved to afford protection against infection with homologous and heterologous viruses.⁸⁴⁻⁸⁹ Furthermore, recombinant FPV vaccines were generated expressing the HA genes of A(H1), A(H7) and A(H9) viruses. An FPV-H1(A/PR/8/34) failed to protect chickens from infection with an A(H7N7) virus,⁹⁰ but vaccination with recombinant FPV expressing the homologous HA gene afforded full protection.^{90,91} Similar results were obtained with a recombinant FPV vaccine expressing the HA gene of avian A(H9N2) influenza virus.^{92,93} Interestingly, co-expression of IL-6 or IL-18 genes improved immunogenicity of FPV-HA vaccines in chickens and ducks.^{85,89}

Recombinant FPV vaccines expressing influenza virus NP and NA genes were tested as well. A recombinant FPV expressing NP failed to protect chickens from infection. However, a FPV-based vaccine expressing both the HA and NP genes simultaneously protected chickens completely from homologous challenge infection.⁹⁴ Notably, recombinant FPV expressing both the HA and NA gene of A(H5N1) virus A/Goose/Guangdong/3/96 protected chickens completely not only against infection with the homologous virus, but also against infection with a virus of another subtype, namely A(H7N1).^{95,96} Although FPV vector vaccines expressing influenza viral antigens rarely have been used in non-avian species, FPV-HA was capable of inducing antibody responses in cats⁹⁷ and afforded protection in pigs when challenged with a low-pathogenic A(H5N2) influenza virus.⁶⁷

Alphavirus vectors

Alphaviruses are single-stranded RNA viruses with a positive sense genome of the *Togaviridae* family. Several alphaviruses are being developed as vaccine vectors, including semliki forest virus (SFV), sindbis virus (SIN) and Venezuelan equine encephalitis (VEE). These vectors often are replication deficient replicons that do not encode viral structural proteins, as these regions of the genome are replaced by transgenes of interest. Viral RNAs are self-replicating and are capable of transgene expression at high levels.⁹⁸ As an added advantage, when using alphavirus replicons pre-existing immunity to the vector should not pose a problem and multiple sequential vaccinations are a possibility.⁹⁹⁻¹⁰² Furthermore, VEE is an appealing vaccine vector, as it was previously shown that VEE mainly targets antigen-presenting cells in the lymphoid tissues and therefore primes rapid and robust immune responses.¹⁰³

SIN, SFV and VEE have all been tested as influenza vaccine vectors (Table 2). An SFV vaccine expressing the HA and NP genes could protect mice from infection with A(H1N1) virus;⁹⁹ the same held true for a SIN replicon expressing either the HA gene or an immunodominant CD8⁺ T-cell NP epitope.^{104,105} VEE was more extensively evaluated as an influenza vaccine vector, VEE-H1(A/PR/8/34) vaccination protected mice from infection with homologous virus,¹⁰¹ even in aged animals.¹⁰⁶ Immunization of chickens with VEE expressing the HA gene of A(H5N1) virus A/HK/156/97 also afforded full protection against this virus.¹⁰⁷ Finally, vaccination of pigs has been studied using different VEE constructs, expressing HA genes of the A(H1) and A(H3) subtypes and an NP gene of an A(H3N2) swine influenza virus. In all cases antibodies were induced by vaccination, and pigs could at least be partially protected from infection with the homologous virus.¹⁰⁸⁻¹¹² Heterosubtypic immunity could be induced when pigs were vaccinated with the VEE-NP construct,¹¹² however VEE-based vaccines performed poorly in the presence of maternal antibodies.¹⁰⁸













A VEE-based candidate vaccine against cytomegalovirus (CMV) has been tested in clinical trials, was shown to be immunogenic, well tolerated and safe in humans.¹¹³ Clinical trials with alphaviruses-based vector influenza vaccines have not yet been conducted, however since these replicons are potentially efficacious (even in the face of pre-existing immunity) and safe, they hold promise as influenza vector vaccines.

Herpes virus vectors

Several recombinant herpes viruses have been tested in animal models as candidate influenza vaccines (Table 2). Duck enteritis virus (DEV), an alphaherpesvirus and the causative agent of duck plague, has caused fatal infections in many species of waterfowl. However, deletion of glycoprotein C (gC) leads to an attenuated DEV that may be exploited for the development of vectored vaccines. Construction of the first DEV-based candidate A(H5N1) influenza vaccine was initially described in 2011.¹¹⁴ Subsequently, it was shown that DEV encoding different HAs of A(H5N1) viruses could protect chickens and ducks from lethal infections with these viruses¹¹⁵⁻¹¹⁷ and was capable of inducing both humoral and cellular immune responses.¹¹⁸










Infectious laryngotracheitis virus (ILT), another alphaherpesvirus that causes disease in mainly poultry, has been tested

Table 2. Overview of vector-based influenza vaccines.

Vector	Model	Antigen	Modification	Subtype	Reference
Alphavirus		HA NP HA/NP	n/a CD8 epitope n/a	H1N1 n/a H1N1	100,103,105 104 98
		HA NP HA/NP	n/a n/a n/a	H1N1, H3N2 H3N2 H3N2	107-111 111 107
		HA	n/a	H5N1	106
Herpesvirus		HA	n/a	H1N1, H3N2, H3N8	130,131,133
		HA HA/NA	n/a n/a	H1N1 H1N1	129,134 128
		HA NA HA/NA	n/a n/a n/a	H5N1, H5N2, H7N1 H5N1 H5N1	115,117-126 120 120
		HA	n/a	H5, H5N1	114
		HA	n/a	H3N8	131
		HA	n/a	H3N8	132
VSV		HA HA NP HA/NP HA/NA	n/a Chimeric n/a n/a n/a	H1N1, H5N1 cH5/1, cH9/1 H1N1 H1N1 H1N1, H5N1	136,139,143,145 140 139 139 140
		HA	n/a	H5N1, H7N1	142
		HA	n/a	H5N1	144

(Continued)

Table 2. (Continued)

Vector	Model	Antigen	Modification	Subtype	Reference
NDV		HA	n/a	H1N1, H5N1	147,158,159,167
		HA	Soluble	H5N1	158
		HA	n/a	H5N1, H5N2, H6, H7, H7N2, H7N9, H9N2	149,150,152,153,155-166
		HA	Soluble	H5N1	158
		HA	Ectodomain	H5N1	151
		HA/NA	n/a	H5N1	155
	HA	n/a	H6	165	
	HA	n/a	H5N1, H5N8	149,154	
		HA	n/a	H5N1	168,169
Baculo		HA	n/a	H1N1, H5N1, H6N8, H7N9, H9N2	171-178
PIV-5		HA	n/a	H3N2, H5N1, H7N9	180-182
		NP	n/a	H5N1	182
		HA	n/a	H7N9	181
		HA/NP	n/a	H7N9	181
	HA	n/a	H3N2	179	

as an influenza vaccine for poultry. Attenuated strains of ILTV expressing HA genes obtained from various A(H5N1) and A(H5N2) influenza virus strains were generated and shown to offer protection against these viruses.^{119,120} However, ILTV should be attenuated sufficiently, as pathogenicity caused by the vector was still observed in a single study.¹¹⁹ ILTV vaccines expressing the NA gene were also generated but were poorly immunogenic; co-expression of HA genes was always required to obtain protective immunity.¹²¹

Another alphaherpesvirus of poultry, turkey herpesvirus (HVT) has also been extensively studied as influenza vaccine vector in chickens. HVT encoding the HA gene of an A(H5N1) virus afforded protection from infection with various A(H5N1) viruses.¹²²⁻¹²⁴ Similarly, a recombinant HVT-H7 vaccine protected chickens against infection with the homologous A(H7N1) virus.¹²⁵ Since chickens are often vaccinated at very

young age (1 day after birth), maternal antibodies against the vector or against the protein encoded by the transgene could influence vaccine efficacy. Interestingly, HVT was shown to be immunogenic even in the presence of these maternal antibodies.¹²⁶ Marek's disease virus (MDV), an alphaherpesvirus closely related to HVT, was shown to be an effective vaccine vector against A(H5N1) virus and even performed better than HVT in a side-by-side comparison in chickens.¹²⁷

Pseudorabiesvirus (PrV) is an alphaherpesvirus of pigs and attenuated strains of PrV have been generated and used for control of Aujeszky's disease in pigs.¹²⁸ Attenuated PrV expressing foreign antigens were generated and are attractive as bivalent vaccines for pigs. The use of PrV-H1 influenza vaccines partially protected pigs from H1N1pdm09 virus infection. Recombinant PrV expressing the NA gene derived from a swine A(H1N1) influenza virus only protected pigs to a limited extent.^{129,130} A recombinant PrV

expressing HA from a swine A(H3N2) virus was tested in mice and induced protective immunity against infection with heterologous A(H3N2) virus.¹³¹

An attenuated strain of equine herpesvirus (EHV-1), an alphaherpesvirus that infects horses, has an impressive safety record in horses and other mammalian species and therefore should be considered an attractive vaccine vector. The HA gene from an equine A(H3N8) influenza virus was cloned into EHV-1 and could induce antibody responses that react with multiple A(H3N8) strains in mice and horses.^{132,133} Interestingly, the protective efficacy of these vaccines was only assessed in dogs, which upon vaccination were partially protected from infection with a canine A(H3N8) influenza virus strain.¹³² In addition, recombinant EHV-1 were constructed that express the HA gene derived from H1N1pdm09 virus. This vaccine candidate afforded mice complete and pigs partial protection from infection.^{134,135}

Herpes viruses encoding influenza virus antigens have mainly been tested as candidate vaccines for poultry in which their protective effectiveness was confirmed. Like other DNA viruses, herpes viruses have a relatively large genome and antigens of interest can easily be cloned into multiple insertion sites. Although an advantage, it also complicates characterization of the vector because the insertion site of the transgene may influence its immunogenicity.¹²²

Vesicular stomatitis virus vectors

Vesicular Stomatitis Virus (VSV) is a rhabdovirus and has a negative sense RNA genome. Candidate influenza vaccines based on VSV have been constructed and have numerous advantages over other vectors. VSV is immunogenic,^{136,137} has a broad tissue tropism and can easily be delivered locally. In contrast to other vectors (like adeno- and poxviruses), there is little evidence for VSV seropositivity in humans, eliminating concerns of pre-existing immunity to the vector. On the other hand, use of VSV as a vector is not without concern: VSV can cause disease in humans¹³⁸ and is known to be neuro-invasive in some species.¹³⁹ Currently, there is no human safety data available for VSV-vectored vaccines and additional experiments are required. An overview of VSV-based influenza vaccines can be found in Table 2.

VSV expressing the HA gene of influenza virus A/WSN/33 (H1N1) proved to be immunogenic in mice and protected the animals from challenge infection.¹³⁷ Since VSV also proved to be pathogenic in mice, most studies with VSV as vector relied on recombinant attenuated VSV viruses with a truncated or deficient G protein. In similar experiments, mice could only be partially protected from infection with influenza virus A/PR/8/34 after vaccination with VSV expressing the corresponding HA gene, whereas expression of only the NP failed to afford protection. However, combination of the HA and NP constructs resulted in full protection from infection.¹⁴⁰ Furthermore, a VSV expressing the HA gene of influenza virus A/Vietnam/1203/04 (H5N1) and the NA gene of influenza virus A/PR/8/34 (H1N1) completely protected mice from infection with a 6:2 reassortant A(H5N1) virus (HA and NA from A/Vietnam/1203/04).¹⁴¹ VSV-H7(A/FPV/Rostock/34) expressing the HA gene of an A(H7N1) virus afforded chickens protection

from developing disease after caused by a virus of the same subtype.¹⁴²

VSV-based candidate A(H5N1) vaccines were constructed and tested in chickens, mice and macaques. Immunization with VSV expressing the HA gene of 2 different avian A(H5N1) viruses completely protected chickens from A(H5N1) virus challenge.¹⁴³ Also in mice and macaques VSV-based A(H5N1) vaccines proved to be immunogenic¹⁴⁴⁻¹⁴⁶ and mice immunized with a recombinant VSV expressing the HA gene of a clade 0 A(H5N1) virus were protected from infection with viruses of the same clade and those of clade 1.^{144,146}

VSV has also been used to construct vaccines that aim at the induction of broadly reactive HA-stalk specific antibodies as a universal influenza vaccine approach. As suggested previously, repeated vaccination with various chimeric HA molecules can boost the induction of stalk-specific antibody responses.¹⁴⁷ Therefore, mice were primed with a recombinant VSV expressing an HA gene with the stalk region of influenza virus A/PR/8/34 and the globular head domain of an A(H9N2) virus. Subsequently mice were boosted with a VSV, expressing an HA gene with the same stalk but with the head domain of an A(H5N1) virus (VSV-cH5/1). As expected, mice could be protected from infection with influenza virus A/PR/8/34 by this vaccination regimen.¹⁴¹ Priming with VSV encoding the full-length HA gene of A/PR/8/34 (H1N1) followed by boost with the VSV-cH9/1 construct also afforded protection against infection with a virus of the A(H5N1) subtype.¹⁴¹ Interestingly, in both experiments it was shown that intra-nasal prime-boost regimens performed better than IM vaccination.

Newcastle disease virus vectors

Newcastle disease virus (NDV) is a single-stranded negative sense RNA paramyxovirus that causes disease in poultry. NDV has several favorable properties as a vaccine vector; no pre-existing immunity in humans exists, NDV can easily be attenuated and reverse genetics systems to rescue recombinant NDV are in place. Thus far, NDV has been extensively characterized as an influenza vaccine vector in poultry, where it serves as a bivalent vaccine capable of inducing immunity against both NDV and influenza virus. As an added advantage, NDV is easily administered to poultry through nasal spray, drinking water or ocular drops. An overview of NDV-based influenza vaccines can be found in Table 2.

The first study using NDV as a vaccine vector for influenza was NDV-H1, that expressed the HA gene of influenza virus A/WSN/1933. Complete protection of mice against homologous challenge infection was achieved, demonstrating that NDV can be used as an influenza vaccine vector.¹⁴⁸ Consequently, a recombinant NDV expressing HA genes of A(H5N1) viruses has been licensed as a poultry vaccine in some countries and was shown to have a protective effect against challenge infection with A(H5N1) viruses in chickens and ducks in various studies.¹⁴⁹⁻¹⁶³ The NDV based A(H5N1) vaccine offered only partial cross-clade protection, but was immunogenic in the presence of maternal antibodies.^{162,163} Expression or co-expression of NA by NDV did not improve immunogenicity in chickens.¹⁵⁶ Also NDVs expressing the HA genes of A(H6), A(H7) and A(H9) subtypes were tested in poultry. Although

most challenge viruses were low-pathogenic, a reduction or complete abrogation of virus shedding could be obtained after inoculation with the respective homologous viruses.^{161,164-167}

To develop NDV-based vaccines for use in humans, their performance has also been tested in mammalian species. In mice, protective immunity against A(H5N1) viruses was induced after vaccination with NDV expressing the homologous HA gene.^{159,160} In one single study, cross-reactive cellular immune responses against A(H1N1) viruses were observed after vaccination with a NDV-H5 construct.¹⁶⁸ The immunogenicity of recombinant NDV expressing the HA and NA genes of influenza virus A/Vietnam/1203/04 (H5N1) was tested in non-human primates. Both constructs induced VN and local IgA antibody responses and afforded protection from A(H5N1) challenge infection.^{169,170} Small numbers of clinical trials have been performed with NDV, which showed that the vector is well tolerated.

Baculovirus vectors

Baculoviruses are extensively used as tool to express and produce influenza virus proteins. Currently, a recombinant HA protein vaccine produced in baculoviruses was approved for human use in the United States. However, baculoviruses have also been explored as live vaccine vectors. Since baculoviruses can readily be manipulated to express foreign antigens and can infect mammalian cells without causing cytopathic effect they are potentially promising vaccine vectors for influenza (Table 2).¹⁷¹

Initially, it was reported that vaccination with recombinant baculovirus expressing the HA gene of influenza virus could induce complete protection from homologous challenge infection.¹⁷² Interestingly, in this study the control group that received an 'empty' baculovirus, not expressing the HA gene, was also protected from challenge infection. Potentially the induction of strong non-specific innate immune responses by vaccination with baculovirus was responsible. Subsequently, several baculoviruses expressing the HA genes of various A (H5N1) influenza viruses were tested in mice and afforded protection against infection with both homologous viruses and A (H5N1) viruses from different clades.^{173,174} Bivalent baculovirus vaccines, expressing 2 different HA genes from A(H5) viruses simultaneously, were also successful in affording cross-clade immunity.^{175,176} Finally, recombinant baculoviruses expressing HA genes of A(H6), A(H7) and A(H9) influenza viruses were capable of inducing protective immunity against infection with homologous viruses in mice.¹⁷⁷⁻¹⁷⁹

Although recombinant baculovirus vector vaccines were tested in mice, efficacy data in other animal models is still lacking. Short-term production of baculovirus-based influenza virus vaccines for use in clinical trials is therefore not likely.

Parainfluenza virus 5 vectors

Parainfluenza virus 5 (PIV-5) is, like NDV, a negative sense RNA paramyxovirus that is only recently being explored as an influenza virus vaccine vector (Table 2). Favorable properties of PIV-5 as a vector include: broad tissue and cell tropism, no clinical disease in humans and availability of reverse genetics

systems. Although PIV-5 does not cause disease in humans, PIV-5 has been associated with 'kennel cough' in dogs.¹⁸⁰

In an initial study, vaccination with PIV-5 expressing the HA of an A(H3) virus afforded protection against homologous challenge infection.¹⁸¹ PIV-5 expressing the HA genes of A/Vietnam/1203/04 (H5N1) and A/Anhui/1/13 (H7N9) also completely protected mice from infection with the homologous influenza virus.^{182,183} PIV-5 expressing an internal protein of influenza virus, in this case the NP gene of A/Vietnam/1203/04, was constructed, but could only partially protect mice from homologous challenge infection. Interestingly, PIV-5 expressing the same NP gene completely protected mice from a heterologous challenge infection with A(H1N1), cellular immune responses targeting NP were the responsible correlate of protection.¹⁸³ Similar results were obtained with recombinant PIV-5-NP(A/Anhui/1/13) in guinea pigs challenged with a homologous A(H7N9) influenza virus.¹⁸²








PIV-5 has been evaluated in mice and guinea pigs, but was not tested as an candidate influenza vaccine in other animal models. Furthermore, clinical trials in humans have not been performed with PIV-5 yet, so safety and efficacy data is therefore not available. Finally, little is known about pre-existing immunity to the vector in humans. However, in dogs, a PIV-5 vector vaccine expressing the HA gene of influenza virus could still induce robust antibody responses in the presence of PIV-5-specific immunity.¹⁸⁰ It remains to be determined whether PIV-5 is safe and immunogenic when used in humans.

Adenovirus vectors

Recombinant adenoviruses (rAd) have attractive properties to serve as vaccine vectors: high titer stocks can be grown, genes of interest can easily be inserted into the stable viral genome, long-term storage at 4 degrees is possible and rAd infects a variety of hosts, tissues and cell types.¹⁸⁴ Furthermore, rAd can even induce robust immune responses when administered orally or intra-nasally, potentially bypassing pre-existing immunity against the vector.¹⁸⁴ Finally, even replication-deficient rAd are known to be immunogenic; adenovirus 5 (Ad5) is a replication-deficient vector that has been evaluated for gene delivery, anti-cancer therapy and as an infectious disease vaccine. An overview of adenovirus-based influenza vaccines can be found in Table 3.

A live adenovirus vaccine that contains 2 different serotypes is already in use for vaccination of humans for decades,¹⁸⁵ indicating that adenoviruses are safe and immunogenic in humans. However, continuation of clinical trials with rAd5 is currently hampered by 2 trial failures: one death was reported after intravenous rAd5 administration,¹⁸⁶ another study showed increased risk of acquiring HIV-1 infection after vaccination with rAd5 expressing HIV-1 genes gag, pol and nef.¹⁸⁷ However, recombinant adenovirus expressing the HA gene of influenza virus A/PR/8/34 proteins proved to be safe and immunogenic in humans, inducing mainly a robust antibody response.¹⁸⁸ A more recent trial in humans with rAd4 expressing the HA gene of an A(H5N1) influenza virus reported enhanced immune responses after co-administration with an HA protein vaccine in the absence of serious adverse events.¹⁸⁹

Table 3. Overview of adenovirus-based influenza vaccines.

Vector	Model	Antigen	Modification	Subtype	Reference
Adenoviruses		HA	n/a	H3N2, H5N1, H7N7, H9N2	191,193-195,199,201-204,206
		HA	Soluble head	H1N1	198
		HA	Glycan shielded	H5N1	207
		NP	n/a	H1N1, H5N1	192,208,209,214
		M2	Consensus	n/a	180
		HA/NP	n/a	H5N1, H7N7, H9N2	206
		HA/NA	n/a	H1N1	211
		NP/M2	n/a	H1N1	216
		NP/M2	Consensus	H1N1	210
		HA/NA/M1	n/a	H1N1, H5N1	213
NP/M1/M2	miRNAs	H1N1	211		
		HA	n/a	H5N1	203,215
		NP	n/a	H5N1	215
		M2	n/a	H5N1	215
		HA/NA	Consensus	H1N1	212
		NP/M2	n/a	H1N1, H5N1	215,216
		HA/NP/M2	n/a	H5N1	215
		HA	n/a	H1N1, H3N2	196,200
		NP	n/a	H3N2	196
		HA/NP	n/a	H3N2	196
		HA	n/a	H5N1, H7N3	202,205
		HA	n/a	H1N1, H5N1	187,188,190
		NP/M1	n/a	H3N2	189
AAV		HA	n/a	H1N1	222
		HA	Broadly neutralizing ab	n/a	223,224
		NP	n/a	H1N1	222
		M1	n/a	H1N1	222
		HA/NP/M1	n/a	H1N1	222
		HA	Broadly neutralizing ab	n/a	224

Finally, a rAd expressing the NP and M1 genes of influenza virus and a rAd5 expressing the HA gene of an A(H1) virus and co-expressing dsRNA as adjuvant were safe and immunogenic in humans.^{190,191}

In addition to the 2 discussed trial failures, a second drawback for the use of rAd in humans is the potential of pre-existing immunity against adenovirus interfering with vaccine efficacy. Currently, as alternatives, non-human adenoviruses^{190,192-194} and low-prevalent adenoviruses¹⁹⁵ are being explored as novel vaccine vectors.

Ad-HA vaccines

Adenoviruses expressing HA genes of a number of different subtypes (A(H1), H3, H5, H7 and H9)) have been tested in various animal models. In the first study with rAd5, a vaccine that expressed the HA gene of an A(H3N2) influenza virus of swine-origin protected mice from challenge infection with a heterologous A(H3N2) virus.¹⁹⁶ A rAd expressing the HA gene

of a different A(H3) virus was shown to be efficacious in pigs,¹⁹⁷ even in the presence of maternal antibodies.¹⁹⁸ Adenovirus vaccines expressing the HA gene of A/PR/8/34, completely protected mice from homologous challenge infection.^{195,199,200} Pigs could also be protected from A/PR/8/34 virus infection by vaccination with rAd5 expressing the HA gene from the H1N1pdm09 virus A/Cal/04/09. Interestingly, pigs were also partially protected by vaccination with this construct from infection with a heterologous A(H1N2) virus.²⁰¹ A rAd expressing the HA gene from A(H5N1), protected mice, chickens and ferrets from infection with the homologous virus,²⁰²⁻²⁰⁴ when the HA gene of A/HK/156/97 was introduced into rAd cross-clade protection was reported.²⁰⁵ The rAd expressing the HA gene of an A(H7) virus was immunogenic in chickens and capable of protecting chickens from homologous challenge infection.²⁰⁶ In 2013, a comprehensive study testing rAd5 vectors expressing the HA genes from avian viruses of the A(H5), A(H7) and A(H9) subtype (and

combinations thereof) showed that mice could be protected from homologous challenge infection. Heterosubtypic immunity was never observed, however it was shown that simultaneous vaccination with 5 different rAd5-HA vaccines was feasible and protected from challenge infection with viruses of all subtypes under investigation.²⁰⁷

Comparable to expressing modified influenza antigens in other vectors with the goal of inducing universal influenza immunity (*i.e.* 'headless' HA, chimeric HA, consensus sequences), a rAd expressing a modified HA gene was constructed. This HA gene was modified to shield the immunodominant head region by glycans to re-direct the immune response from the HA head region to target the more conserved stalk region and afford broad protection. Indeed, a rAd expressing the HA gene of an A(H5) influenza virus, either wildtype or glycosylated, afforded cross-clade protection in mice, the glycosylated HA performed better than its wildtype counterpart.²⁰⁸ Heterosubtypic immunity with these glycan-shielded constructs has not been reported yet.

Ad-NP, Ad-M1, Ad-M2 and Ad-NA vaccines

Different vaccination regimens with rAd constructs expressing the NP gene were tested in animal models with some reports of heterosubtypic immunity. A rAdC7 expressing the NP gene of A/PR/8/34 could partially protect mice from infection with some - but not all - influenza viruses of the A(H5N1) subtype.¹⁹³ Vice versa, rAd expressing the NP gene of an avian A(H5N1) virus completely protected mice from infection with A(H1N1) virus.²⁰⁹ However, vaccination of pigs with a comparable rAd5-NP construct could not afford protection from homologous challenge infection, whereas addition of a rAd5-HA construct to the vaccine cocktail completely restored the protective capacities.¹⁹⁷

Vaccination of mice with a combination rAd5 vaccine, including constructs expressing both the NP and M2 genes, protected mice from homologous challenge infection.²¹⁰ A rAd5 expressing an M2 consensus sequence could even afford protection from infection with various A(H1N1) influenza viruses¹⁸¹ and abrogated contact transmission to sentinel mice.²¹¹ When both NP and M1 consensus gene sequences were expressed by rAd, vaccination led to partial immunity to A(H1N1) virus infection in mice. A similar rAd vaccine, expressing the NP and M1 genes from an A(H3N2) virus, induced T-cell responses and proved to be safe in humans.¹⁹⁰ Recently, a novel approach was tested when rAdC68 was constructed to express miRNAs that target NP, M1 and M2 RNA from A/PR/8/34 influenza virus, and these constructs protected mice from A(H1N1), A(H5N1) and A(H9N2) challenge infection.²¹²

A bivalent rAd5 vaccine, expressing the HA and NA consensus gene sequences of multiple H1N1pdm09 viruses protected both mice and ferrets from infection with H1N1pdm09 influenza virus.²¹³ A trivalent vaccine expressing the M1 gene in addition to the HA and NA genes from either 1918 pandemic A(H1N1) or avian influenza A(H5N1) protected mice from challenge infection with A(H5N1) viruses from different clades. Taking this one step further, pentavalent vaccines that expressed the HA, NA and M1 genes from avian A(H5N1) and the HA and NA genes from 1918

pandemic A(H1N1) performed superior in inducing protection from A(H5N1) infection.²¹⁴

Adenovirus heterologous prime boost regimens

Recombinant Ad5 was extensively tested in heterologous prime boost vaccination regimens, in which animals were primed with DNA encoding the HA, NP and/or M2 genes and subsequently boosted with rAd5 expressing the same proteins. DNA priming followed by rAd5-NP(A/PR/8/34) boost vaccination completely protected mice from homologous challenge infection and afforded heterosubtypic immunity upon infection with A(H3N2) and some A(H5N1) influenza strains.²¹⁵ On the contrary, ferrets could not be protected from A(H5N1) infection by this vaccination regimen.²¹⁶ Similar negative results were obtained in ferrets with rAd constructs expressing the M2 gene.²¹⁶ In heterologous prime boost regimens where DNA vaccination was followed by vaccination with a bivalent rAd5 construct expressing both the M2 and NP genes, mice were completely protected from infection with A(H1N1) and A(H5N1),²¹⁷ but conflicting results were again obtained in ferrets.^{216,217} Recombinant Ad5 expressing the HA gene was always protective in ferrets when a DNA prime followed by rAd5 boost regimen was used, inducing protection against infection with the homologous virus in all cases.²¹⁶

Adeno-associated virus vectors

Adeno-associated virus (AAV) is a parvovirus that is replication-deficient in humans. Like adenovirus, AAV has a broad cell, tissue and host tropism and therefore is a potential good vector vaccine.²¹⁸ However, drawbacks of using AAV include: limited capacity for transgenes, presence of pre-existing immunity in humans and the technical challenge of producing high titer stocks. Initially, AAV was not explored as a vaccine vector as it was considered to be poorly immunogenic, however vaccination studies in mice showed that AAV-2 expressing an HSV-2 glycoprotein was immunogenic and a potent inducer of T-cell and antibody responses,²¹⁹ and currently modifications are being made to AAV to increase immunogenicity.²²⁰

A limited number of studies evaluating AAV as a vector for influenza vaccination has been performed (Table 3). Initially, an AAV expressing the HA gene or NP gene was shown to be protective in mice.^{221,222} A more recent study tested AAV vaccines expressing the HA, NP or M1 genes of H1N1pdm09 in mice. Whereas AAV-HA afforded full protection from H1N1pdm09 infection, AAV-NP protected mice partially and AAV-M1 did not afford protection. Simultaneous vaccination with all 3 constructs afforded protection from homologous challenge infection.²²³ Recently, in an alternative vaccination approach, AAV was constructed to express a transgene encoding a influenza virus-specific broadly neutralizing antibody. AAV constructs expressing the broadly neutralizing antibody 'F10' protected mice from infection with 3 different A(H1N1) strains,²²⁴ whereas AAV expressing the broadly neutralizing antibody 'FI6' protected mice and ferrets from infection with various A(H5N1) and A(H1N1) viruses.²²⁵

Conclusions

Viral vectors have potential as novel vaccine candidates in times of pressing need for game-changing vaccines that induce broadly protective immunity against a wide variety of influenza viruses. The major advantage of viral vectors is the possibility of expressing any foreign antigen with or without modification *in vivo*. Since the proteins are expressed in their native confirmation, antibody responses of the desired specificities are induced. In addition, viral vectors allow *de novo* protein synthesis in the cytoplasm of infected cells facilitating endogenous antigen processing and MHC class I presentation of immunogenic peptides, which is a requirement for the efficient induction of virus-specific CD8⁺ T-cell responses. Although all vectors discussed have their own respective advantages and disadvantages, most are replication-deficient in mammalian host cells and are therefore safe for human use, even in immunocompromised individuals. Pre-existing immunity to the vector may pose a problem for some vectors, however there are viral vectors available (like VSV) for which the human population is immunologically naïve. Other vectors (like MVA) proved to be immunogenic even in the presence of pre-existing immunity. For some vector technologies there are some safety concerns, like the use of herpes viruses that persistently infect their hosts and DNA vaccines that might integrate into the host genome. These properties might restrict their applicability as prophylactic vaccines.

As discussed in this review, viral vectors as potential influenza vaccine candidates were not only evaluated in animal models and humans, they were also extensively tested in influenza A virus reservoir species. Vaccination of reservoir species could potentially limit transmission of avian and swine influenza A virus transmission, and therefore limit the zoonotic transmission of these potential (pre-)pandemic viruses to the human host.

In the future, more novel vector-based influenza candidate vaccines will be developed and tested in clinical trials. There is potential for improvement by the modification of viral antigens, like the 'headless' or 'shielded' HA constructs, to broaden the reactivity of vaccine induced antibodies. In addition to modifying influenza virus antigens, post-translational modifications and modifications to promoter sequences could also alter and improve the immunogenicity.^{226,227} The biggest challenge of taking vector-based vaccines to the market may be obtaining approval from the regulatory authorities. Only when their safety and superiority over existing vaccine formulations have been demonstrated, implementation of these novel vector-based vaccines may be considered.

Abbreviations

TIV	Trivalent inactivated vaccine
QIV	Quadrivalent inactivated vaccine
LAIV	Live-attenuated influenza vaccine
IM	Intra-muscular
HA	Hemagglutinin
NA	Neuraminidase
CTL	Cytotoxic T-lymphocyte
VV	Vaccinia virus

MVA	Modified vaccinia virus Ankara
BSL-1	Biosafety level 1
NP	Nucleoprotein
VN	Virus neutralizing
HI	Hemagglutination inhibition
M1	Matrix 1
rAd	Recombinant adenovirus
RCN	Raccoonpox virus
CNPV	Canarypox virus
FPV	Fowlpox virus
SFV	Semliki forest virus
SIN	Sindbis virus
VEE	Venezuelan equine encephalitis
CMV	Cytomegalovirus
DEV	Duck enteritis virus
gC	Glycoprotein C
ILTV	Infectious laryngotracheitis virus
HVT	Turkey herpesvirus
MDV	Marek's disease virus
PrV	Pseudorabies virus
EHV-1	Equine herpesvirus
VSV	Vesicular stomatitis virus
NDV	Newcastle disease virus
PIV-5	Parainfluenza virus 5
AAV	Adeno-associated virus

Disclosure of potential conflicts of interest

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References

- [1] Murphy BR, Kasel JA, Chanock RM. Association of serum anti-neuraminidase antibody with resistance to influenza in man. *N Engl J Med* 1972; 286:1329-32; PMID:5027388; <http://dx.doi.org/10.1056/NEJM197206222862502>
- [2] Nichol KL. Live attenuated influenza virus vaccines: new options for the prevention of influenza. *Vaccine* 2001; 19:4373-7; PMID:11483261; [http://dx.doi.org/10.1016/S0264-410X\(01\)00143-8](http://dx.doi.org/10.1016/S0264-410X(01)00143-8)
- [3] Gilca R, Skowronski DM, Douville-Fradet M, Amini R, Boulianne N, Rouleau I, Martineau C, Charest H, De Serres G. Mid-Season estimates of influenza vaccine effectiveness against influenza A (H3N2) hospitalization in the elderly in Quebec, Canada, January 2015. *PLoS One* 2015; 10:e0132195; PMID:26200655; <http://dx.doi.org/10.1371/journal.pone.0132195>
- [4] Pebody RG, Warburton F, Ellis J, Andrews N, Thompson C, von Wissmann B, Green HK, Cottrell S, Johnston J, de Lusignan S, et al. Low effectiveness of seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2014/15 mid-season results. *Euro Surveill* 2015; 20:21025; PMID:25677050; <http://dx.doi.org/10.2807/1560-7917.ES2015.20.5.21025>
- [5] Skowronski DM, Chambers C, Sabaiduc S, De Serres G, Dickinson JA, Winter AL, Drews SJ, Fonseca K, Charest H, Gubbay JB, et al. Interim estimates of 2014/15 vaccine effectiveness against influenza

- A(H3N2) from Canada's Sentinel Physician Surveillance Network, January 2015. *Euro Surveill* 2015; 20(4):21022; PMID:25655053; <http://dx.doi.org/10.2807/1560-7917.ES2015.20.4.21022>
- [6] Yang ZF, Mok CK, Peiris JS, Zhong NS. Human Infection with a Novel Avian Influenza A(H5N6) Virus. *N Engl J Med* 2015; 373:487-9; PMID:26222578; <http://dx.doi.org/10.1056/NEJMc1502983>
- [7] Fouchier RA, Schneeberger PM, Rozendaal FW, Broekman JM, Kemink SA, Munster V, Kuiken T, Rimmelzwaan GF, Schutten M, Van Doornum GJ, et al. Avian influenza A virus (H7N9) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proc Natl Acad Sci U S A* 2004; 101:1356-61; PMID:14745020; <http://dx.doi.org/10.1073/pnas.0308352100>
- [8] Gao R, Cao B, Hu Y, Feng Z, Wang D, Hu W, Chen J, Jie Z, Qiu H, Xu K, et al. Human infection with a novel avian-origin influenza A (H7N9) virus. *N Engl J Med* 2013; 368:1888-97; PMID:23577628; <http://dx.doi.org/10.1056/NEJMoa1304459>
- [9] Butt KM, Smith GJ, Chen H, Zhang LJ, Leung YH, Xu KM, Lim W, Webster RG, Yuen KY, Peiris JS, et al. Human infection with an avian H9N2 influenza A virus in Hong Kong in 2003. *J Clin Microbiol* 2005; 43:5760-7; PMID:16272514; <http://dx.doi.org/10.1128/JCM.43.11.5760-5767.2005>
- [10] Chen H, Yuan H, Gao R, Zhang J, Wang D, Xiong Y, Fan G, Yang F, Li X, Zhou J, et al. Clinical and epidemiological characteristics of a fatal case of avian influenza A H10N8 virus infection: a descriptive study. *Lancet* 2014; 383:714-21; PMID:24507376; [http://dx.doi.org/10.1016/S0140-6736\(14\)60111-2](http://dx.doi.org/10.1016/S0140-6736(14)60111-2)
- [11] Nicholson KG, Colegate AE, Podda A, Stephenson I, Wood J, Ypma E, Zambon MC. Safety and antigenicity of non-adjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a randomised trial of two potential vaccines against H5N1 influenza. *Lancet* 2001; 357:1937-43; PMID:11425416; [http://dx.doi.org/10.1016/S0140-6736\(00\)05066-2](http://dx.doi.org/10.1016/S0140-6736(00)05066-2)
- [12] Broadbent AJ, Subbarao K. Influenza virus vaccines: lessons from the 2009 H1N1 pandemic. *Curr Opin Virol* 2011; 1:254-62; PMID:22125588; <http://dx.doi.org/10.1016/j.coviro.2011.08.002>
- [13] Mackett M, Smith GL, Moss B. Vaccinia virus: a selectable eukaryotic cloning and expression vector. *Biotechnol* 1992; 24:495-9
- [14] Panicali D, Paoletti E. Construction of poxviruses as cloning vectors: insertion of the thymidine kinase gene from herpes simplex virus into the DNA of infectious vaccinia virus. *Proc Natl Acad Sci U S A* 1982; 79:4927-31; PMID:6289324; <http://dx.doi.org/10.1073/pnas.79.16.4927>
- [15] Smith GL, Murphy BR, Moss B. Construction and characterization of an infectious vaccinia virus recombinant that expresses the influenza hemagglutinin gene and induces resistance to influenza virus infection in hamsters. *Proc Natl Acad Sci U S A* 1983; 80:7155-9; PMID:6580632; <http://dx.doi.org/10.1073/pnas.80.23.7155>
- [16] Smith GL, Levin JZ, Palese P, Moss B. Synthesis and cellular location of the ten influenza polypeptides individually expressed by recombinant vaccinia viruses. *Virology* 1987; 160:336-45; PMID:3310381; [http://dx.doi.org/10.1016/0042-6822\(87\)90004-3](http://dx.doi.org/10.1016/0042-6822(87)90004-3)
- [17] Mayr A, Munz E. Changes in the vaccinia virus through continuing passages in chick embryo fibroblast cultures. *Zentralbl Bakteriol Orig* 1964; 195:24-35; PMID:5890664
- [18] Mayr A, Stickl H, Muller HK, Danner K, Singer H. The smallpox vaccination strain MVA: marker, genetic structure, experience gained with the parental vaccination and behavior in organisms with a debilitated defence mechanism (author's transl). *Zentralbl Bakteriol B* 1978; 167:375-90; PMID:219640
- [19] Antoine G, Scheiflinger F, Dorner F, Falkner FG. The complete genomic sequence of the modified vaccinia Ankara strain: comparison with other orthopoxviruses. *Virology* 1998; 244:365-96; PMID:9601507; <http://dx.doi.org/10.1006/viro.1998.9123>
- [20] Meisinger-Henschel C, Spath M, Lukassen S, Wolferstatter M, Kachelriess H, Baur K, Dirmeier U, Wagner M, Chaplin P, Suter M, et al. Introduction of the six major genomic deletions of modified vaccinia virus Ankara (MVA) into the parental vaccinia virus is not sufficient to reproduce an MVA-like phenotype in cell culture and in mice. *J Virol* 2010; 84:9907-19; PMID:20668072; <http://dx.doi.org/10.1128/JVI.00756-10>
- [21] Meyer H, Sutter G, Mayr A. Mapping of deletions in the genome of the highly attenuated vaccinia virus MVA and their influence on virulence. *J Gen Virol* 1991; 72(5):1031-8; PMID:2033387; <http://dx.doi.org/10.1099/0022-1317-72-5-1031>
- [22] Sutter G, Moss B. Nonreplicating vaccinia vector efficiently expresses recombinant genes. *Proc Natl Acad Sci U S A* 1992; 89:10847-51; PMID:1438287; <http://dx.doi.org/10.1073/pnas.89.22.10847>
- [23] Stickl HA. Smallpox vaccination and its consequences: first experiences with the highly attenuated smallpox vaccine "MVA." *Prev Med* 1974; 3:97-101; PMID:4205586; [http://dx.doi.org/10.1016/0091-7435\(74\)90066-8](http://dx.doi.org/10.1016/0091-7435(74)90066-8)
- [24] Ramirez JC, Gherardi MM, Esteban M. Biology of attenuated modified vaccinia virus Ankara recombinant vector in mice: virus fate and activation of B- and T-cell immune responses in comparison with the Western Reserve strain and advantages as a vaccine. *J Virol* 2000; 74:923-33; PMID:10623755; <http://dx.doi.org/10.1128/JVI.74.2.923-933.2000>
- [25] Stittelaar KJ, Wyatt LS, de Swart RL, Vos HW, Groen J, van Amerongen G, van Binnendijk RS, Rozenblatt S, Moss B, Osterhaus AD. Protective immunity in macaques vaccinated with a modified vaccinia virus Ankara-based measles virus vaccine in the presence of passively acquired antibodies. *J Virol* 2000; 74:4236-43; PMID:10756037; <http://dx.doi.org/10.1128/JVI.74.9.4236-4243.2000>
- [26] Veits J, Romer-Oberdorfer A, Helferich D, Durban M, Suezter Y, Sutter G, Mettenleiter TC. Protective efficacy of several vaccines against highly pathogenic H5N1 avian influenza virus under experimental conditions. *Vaccine* 2008; 26:1688-96; PMID:18291561; <http://dx.doi.org/10.1016/j.vaccine.2008.01.016>
- [27] Werner GT, Jentsch U, Metzger E, Simon J. Studies on poxvirus infections in irradiated animals. *Arch Virol* 1980; 64:247-56; PMID:6250514; <http://dx.doi.org/10.1007/BF01322704>
- [28] Blanchard TJ, Alcamì A, Andrea P, Smith GL. Modified vaccinia virus Ankara undergoes limited replication in human cells and lacks several immunomodulatory proteins: implications for use as a human vaccine. *J Gen Virol* 1998; 79 (Pt 5):1159-67; PMID:9603331; <http://dx.doi.org/10.1099/0022-1317-79-5-1159>
- [29] Buttner M, Czerny CP, Lehner KH, Wertz K. Interferon induction in peripheral blood mononuclear leukocytes of man and farm animals by poxvirus vector candidates and some poxvirus constructs. *Vet Immunol Immunopathol* 1995; 46:237-50; PMID:7502485; [http://dx.doi.org/10.1016/0165-2427\(94\)05357-X](http://dx.doi.org/10.1016/0165-2427(94)05357-X)
- [30] Delaloye J, Roger T, Steiner-Tardivel QG, Le Roy D, Knaup Reymond M, Akira S, Petrilli V, Gomez CE, Perdiguer B, Tschopp J, et al. Innate immune sensing of modified vaccinia virus Ankara (MVA) is mediated by TLR2-TLR6, MDA-5 and the NALP3 inflammasome. *PLoS Pathog* 2009; 5:e1000480; PMID:19543380; <http://dx.doi.org/10.1371/journal.ppat.1000480>
- [31] Fleige H, Ravens S, Moschovakis GL, Bolter J, Willenzon S, Sutter G, Haussler S, Kalinke U, Prinz I, Forster R. IL-17-induced CXCL12 recruits B cells and induces follicle formation in BALT in the absence of differentiated FDCs. *J Exp Med* 2014; 211:643-51; PMID:24663215; <http://dx.doi.org/10.1084/jem.20131737>
- [32] Forster R, Wolf G, Mayr A. Highly attenuated poxviruses induce functional priming of neutrophils in vitro. *Arch Virol* 1994; 136:219-26; PMID:8002789; <http://dx.doi.org/10.1007/BF01538831>
- [33] Halle S, Dujardin HC, Bakocevic N, Fleige H, Danzer H, Willenzon S, Suezter Y, Hammerling G, Garbi N, Sutter G, et al. Induced bronchus-associated lymphoid tissue serves as a general priming site for T cells and is maintained by dendritic cells. *J Exp Med* 2009; 206:2593-601; PMID:19917776; <http://dx.doi.org/10.1084/jem.20091472>
- [34] Lehmann MH, Kastenmuller W, Kandemir JD, Brandt F, Suezter Y, Sutter G. Modified vaccinia virus ankara triggers chemotaxis of monocytes and early respiratory immigration of leukocytes by induction of CCL2 expression. *J Virol* 2009; 83:2540-52; PMID:19129447; <http://dx.doi.org/10.1128/JVI.01884-08>
- [35] Waibler Z, Anzaghe M, Ludwig H, Akira S, Weiss S, Sutter G, Kalinke U. Modified vaccinia virus Ankara induces Toll-like receptor-independent type I interferon responses. *J Virol* 2007; 81:12102-10; PMID:17855554; <http://dx.doi.org/10.1128/JVI.0-1190-07>

- [36] Altenburg AF, Kreijtz JH, de Vries RD, Song F, Fux R, Rimmelzwaan GF, Sutter G, Volz A. Modified vaccinia virus ankara (MVA) as production platform for vaccines against influenza and other viral respiratory diseases. *Viruses* 2014; 6:2735-61; PMID:25036462; <http://dx.doi.org/10.3390/v6072735>
- [37] Sutter G, Wyatt LS, Foley PL, Bennink JR, Moss B. A recombinant vector derived from the host range-restricted and highly attenuated MVA strain of vaccinia virus stimulates protective immunity in mice to influenza virus. *Vaccine* 1994; 12:1032-40; PMID:7975844; [http://dx.doi.org/10.1016/0264-410X\(94\)90341-7](http://dx.doi.org/10.1016/0264-410X(94)90341-7)
- [38] Castrucci MR, Facchini M, Di Mario G, Garulli B, Sciaraffia E, Meola M, Fabiani C, De Marco MA, Cordioli P, Siccardi A, et al. Modified vaccinia virus Ankara expressing the hemagglutinin of pandemic (H1N1) 2009 virus induces cross-protective immunity against Eurasian 'avian-like' H1N1 swine viruses in mice. *Influenza Other Respir Viruses* 2014; 8:367-75; PMID:24373385; <http://dx.doi.org/10.1111/irv.12221>
- [39] Hessel A, Schwendinger M, Fritz D, Coulibaly S, Holzer GW, Sabarth N, Kistner O, Wodal W, Kerschbaum A, Savidis-Dacho H, et al. A pandemic influenza H1N1 live vaccine based on modified vaccinia Ankara is highly immunogenic and protects mice in active and passive immunizations. *PLoS One* 2010; 5:e12217; PMID:20808939; <http://dx.doi.org/10.1371/journal.pone.0012217>
- [40] Kreijtz JH, Suzer Y, Bodewes R, Schwantes A, van Amerongen G, Verburgh RJ, de Mutsert G, van den Brand J, van Trierum SE, Kuiken T, et al. Evaluation of a modified vaccinia virus Ankara (MVA)-based candidate pandemic influenza A/H1N1 vaccine in the ferret model. *J Gen Virol* 2010; 91:2745-52; PMID:20719991; <http://dx.doi.org/10.1099/vir.0.024885-0>
- [41] Florek NW, Weinfurter JT, Jegaskanda S, Brewoo JN, Powell TD, Young GR, Das SC, Hatta M, Broman KW, Hungnes O, et al. Modified vaccinia virus Ankara encoding influenza virus hemagglutinin induces heterosubtypic immunity in macaques. *J Virol* 2014; 88:13418-28; PMID:25210172; <http://dx.doi.org/10.1128/JVI.01219-14>
- [42] de Jong JC, Claas EC, Osterhaus AD, Webster RG, Lim WL. A pandemic warning? *Nature* 1997; 389:554; PMID:9335492; <http://dx.doi.org/10.1038/39218>
- [43] Herfst S, Schrauwen EJ, Linster M, Chutinimitkul S, de Wit E, Munster VJ, Sorrell EM, Bestebroer TM, Burke DF, Smith DJ, et al. Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 2012; 336:1534-41; PMID:22723413; <http://dx.doi.org/10.1126/science.1213362>
- [44] Kreijtz JH, Suezter Y, de Mutsert G, van Amerongen G, Schwantes A, van den Brand JM, Fouchier RA, Lower J, Osterhaus AD, Sutter G, et al. MVA-based H5N1 vaccine affords cross-clade protection in mice against influenza A/H5N1 viruses at low doses and after single immunization. *PLoS One* 2009; 4:e7790; PMID:19915662; <http://dx.doi.org/10.1371/journal.pone.0007790>
- [45] Kreijtz JH, Suezter Y, van Amerongen G, de Mutsert G, Schnierle BS, Wood JM, Kuiken T, Fouchier RA, Lower J, Osterhaus AD, et al. Recombinant modified vaccinia virus Ankara-based vaccine induces protective immunity in mice against infection with influenza virus H5N1. *J Infect Dis* 2007; 195:1598-606; PMID:17471429; <http://dx.doi.org/10.1086/517614>
- [46] Hessel A, Schwendinger M, Holzer GW, Orlinger KK, Coulibaly S, Savidis-Dacho H, Zips ML, Crowe BA, Kreil TR, Ehrlich HJ, et al. Vectors based on modified vaccinia Ankara expressing influenza H5N1 hemagglutinin induce substantial cross-clade protective immunity. *PLoS One* 2011; 6:e16247; PMID:21283631; <http://dx.doi.org/10.1371/journal.pone.0016247>
- [47] Kreijtz JH, Suezter Y, de Mutsert G, van den Brand JM, van Amerongen G, Schnierle BS, Kuiken T, Fouchier RA, Lower J, Osterhaus AD, et al. Recombinant modified vaccinia virus Ankara expressing the hemagglutinin gene confers protection against homologous and heterologous H5N1 influenza virus infections in macaques. *J Infect Dis* 2009; 199:405-13; PMID:19061423; <http://dx.doi.org/10.1086/595984>
- [48] Kreijtz JH, Goeijenbier M, Moesker FM, van den Dries L, Goeijenbier S, De Gruyter HL, Lehmann MH, Mutsert G, van de Vijver DA, Volz A, et al. Safety and immunogenicity of a modified-vaccinia-virus-Ankara-based influenza A H5N1 vaccine: a randomised, double-blind phase 1/2a clinical trial. *Lancet Infect Dis* 2014; 14:1196-207; PMID:25455987; [http://dx.doi.org/10.1016/S1473-3099\(14\)70963-6](http://dx.doi.org/10.1016/S1473-3099(14)70963-6)
- [49] de Vries RD, De Gruyter HL, Bestebroer TM, Pronk M, Fouchier RA, Osterhaus AD, Sutter G, Kreijtz JH, Rimmelzwaan GF. Induction of influenza (H5N8) antibodies by modified vaccinia virus Ankara H5N1 vaccine. *Emerg Infect Dis* 2015; 21:1086-8; PMID:25988813; <http://dx.doi.org/10.3201/eid2106.150021>
- [50] Breathnach CC, Clark HJ, Clark RC, Olsen CW, Townsend HG, Lunn DP. Immunization with recombinant modified vaccinia Ankara (rMVA) constructs encoding the HA or NP gene protects ponies from equine influenza virus challenge. *Vaccine* 2006; 24:1180-90; PMID:16194586; <http://dx.doi.org/10.1016/j.vaccine.2005.08.091>
- [51] Kreijtz JH, Wiersma LC, De Gruyter HL, Vogelzang-van Trierum SE, van Amerongen G, Stittelaar KJ, Fouchier RA, Osterhaus AD, Sutter G, Rimmelzwaan GF. A single immunization with modified vaccinia virus Ankara-based influenza virus H7 vaccine affords protection in the influenza A(H7N9) pneumonia ferret model. *J Infect Dis* 2015; 211:791-800; PMID:25246535; <http://dx.doi.org/10.1093/infdis/jiu528>
- [52] Hessel A, Savidis-Dacho H, Coulibaly S, Portsmouth D, Kreil TR, Crowe BA, Schwendinger MG, Pilz A, Barrett PN, Falkner FG, et al. MVA vectors expressing conserved influenza proteins protect mice against lethal challenge with H5N1, H9N2 and H7N1 viruses. *PLoS One* 2014; 9:e88340; PMID:24523886; <http://dx.doi.org/10.1371/journal.pone.0088340>
- [53] Bender BS, Rowe CA, Taylor SF, Wyatt LS, Moss B, Small PA, Jr. Oral immunization with a replication-deficient recombinant vaccinia virus protects mice against influenza. *J Virol* 1996; 70:6418-24; PMID:8709274
- [54] Brewoo JN, Powell TD, Jones JC, Gundlach NA, Young GR, Chu H, Das SC, Partidos CD, Stinchcomb DT, Osorio JE. Cross-protective immunity against multiple influenza virus subtypes by a novel modified vaccinia Ankara (MVA) vectored vaccine in mice. *Vaccine* 2013; 31:1848-55; PMID:23376279; <http://dx.doi.org/10.1016/j.vaccine.2013.01.038>
- [55] Poon LL, Leung YH, Nicholls JM, Perera PY, Lichy JH, Yamamoto M, Waldmann TA, Peiris JS, Perera LP. Vaccinia virus-based multi-valent H5N1 avian influenza vaccines adjuvanted with IL-15 confer sterile cross-clade protection in mice. *J Immunol* 2009; 182:3063-71; PMID:19234203; <http://dx.doi.org/10.4049/jimmunol.0803467>
- [56] Boyd AC, Ruiz-Hernandez R, Peroval MY, Carson C, Balkissoon D, Staines K, Turner AV, Hill AV, Gilbert SC, Butter C. Towards a universal vaccine for avian influenza: protective efficacy of modified Vaccinia virus Ankara and Adenovirus vaccines expressing conserved influenza antigens in chickens challenged with low pathogenic avian influenza virus. *Vaccine* 2013; 31:670-5; PMID:23200938; <http://dx.doi.org/10.1016/j.vaccine.2012.11.047>
- [57] Lambe T, Carey JB, Li Y, Spencer AJ, van Laarhoven A, Mullarkey CE, Vrdoljak A, Moore AC, Gilbert SC. Immunity against heterosubtypic influenza virus induced by adenovirus and MVA expressing nucleoprotein and matrix protein-1. *Sci Rep* 2013; 3:1443; PMID:23485942; <http://dx.doi.org/10.1038/srep01443>
- [58] Mullarkey CE, Boyd A, van Laarhoven A, Lefevre EA, Veronica Carr B, Baratelli M, Molesti E, Temperton NJ, Butter C, Charleston B, et al. Improved adjuvanting of seasonal influenza vaccines: pre-clinical studies of MVA-NP+M1 coadministration with inactivated influenza vaccine. *Eur J Immunol* 2013; 43:1940-52; PMID:235-89155; <http://dx.doi.org/10.1002/eji.201242922>
- [59] Powell TJ, Peng Y, Berthoud TK, Blais ME, Lillie PJ, Hill AV, Rowland-Jones SL, McMichael AJ, Gilbert SC, Dong T. Examination of influenza specific T cell responses after influenza virus challenge in individuals vaccinated with MVA-NP+M1 vaccine. *PLoS One* 2013; 8:e62778; PMID:23658773; <http://dx.doi.org/10.1371/journal.pone.0062778>
- [60] Berthoud TK, Hamill M, Lillie PJ, Hwenda L, Collins KA, Ewer KJ, Milicic A, Poyntz HC, Lambe T, Fletcher HA, et al. Potent CD8+ T-cell immunogenicity in humans of a novel heterosubtypic

- influenza A vaccine, MVA-NP+M1. *Clin Infect Dis* 2011; 52:1-7; PMID:21148512; <http://dx.doi.org/10.1093/cid/ciq015>
- [61] Lillie PJ, Berthoud TK, Powell TJ, Lambe T, Mullarkey C, Spencer AJ, Hamill M, Peng Y, Blais ME, Duncan CJ, et al. Preliminary assessment of the efficacy of a T-cell-based influenza vaccine, MVA-NP+M1, in humans. *Clin Infect Dis* 2012; 55:19-25; PMID:22441650; <http://dx.doi.org/10.1093/cid/cis327>
- [62] Antrobus RD, Lillie PJ, Berthoud TK, Spencer AJ, McLaren JE, Ladell K, Lambe T, Milicic A, Price DA, Hill AV, et al. A T cell-inducing influenza vaccine for the elderly: safety and immunogenicity of MVA-NP+M1 in adults aged over 50 years. *PLoS One* 2012; 7:e48322; PMID:23118984; <http://dx.doi.org/10.1371/journal.pone.0048322>
- [63] Antrobus RD, Berthoud TK, Mullarkey CE, Hoschler K, Coughlan L, Zambon M, Hill AV, Gilbert SC. Coadministration of seasonal influenza vaccine and MVA-NP+M1 simultaneously achieves potent humoral and cell-mediated responses. *Mol Ther* 2014; 22:233-8; PMID:23831594; <http://dx.doi.org/10.1038/mt.2013.162>
- [64] Kamlangdee A, Kingstad-Bakke B, Anderson TK, Goldberg TL, Osorio JE. Broad protection against avian influenza virus by using a modified vaccinia Ankara virus expressing a mosaic hemagglutinin gene. *J Virol* 2014; 88:13300-9; PMID:25210173; <http://dx.doi.org/10.1128/JVI.01532-14>
- [65] de Vries RD, Altenburg AF, Rimmelzwaan GF. Universal influenza vaccines, science fiction or soon reality? *Expert Rev Vaccines* 2015; 14:1299-301; PMID:26104835; <http://dx.doi.org/10.1586/14760584.2015.1060860>
- [66] Tartaglia J, Perkus ME, Taylor J, Norton EK, Audonnet JC, Cox WI, Davis SW, van der Hoeven J, Meignier B, Riviere M, et al. NYVAC: a highly attenuated strain of vaccinia virus. *Virology* 1992; 188:217-32; PMID:1566575; [http://dx.doi.org/10.1016/0042-6822\(92\)90752-B](http://dx.doi.org/10.1016/0042-6822(92)90752-B)
- [67] Kyriakis CS, De Vleeschauwer A, Barbe F, Bublot M, Van Reeth K. Safety, immunogenicity and efficacy of poxvirus-based vector vaccines expressing the haemagglutinin gene of a highly pathogenic H5N1 avian influenza virus in pigs. *Vaccine* 2009; 27:2258-64; PMID:19428840; <http://dx.doi.org/10.1016/j.vaccine.2009.02.006>
- [68] Kingstad-Bakke B, Kamlangdee A, Osorio JE. Mucosal administration of raccoonpox virus expressing highly pathogenic avian H5N1 influenza neuraminidase is highly protective against H5N1 and seasonal influenza virus challenge. *Vaccine* 2015; 33:5155-62; PMID:26271828; <http://dx.doi.org/10.1016/j.vaccine.2015.08.005>
- [69] Kingstad-Bakke B, Brewoo JN, Mai le Q, Kawaoka Y, Osorio JE. Effects of route and coadministration of recombinant raccoon poxviruses on immune responses and protection against highly pathogenic avian influenza in mice. *Vaccine* 2012; 30:6402-8; PMID:22921740; <http://dx.doi.org/10.1016/j.vaccine.2012.08.018>
- [70] Adams AA, Sturgill TL, Breathnach CC, Chambers TM, Siger L, Minke JM, Horohov DW. Humoral and cell-mediated immune responses of old horses following recombinant canarypox virus vaccination and subsequent challenge infection. *Vet Immunol Immunopathol* 2011; 139:128-40; PMID:21035197; <http://dx.doi.org/10.1016/j.vetimm.2010.09.006>
- [71] Soboll G, Breathnach CC, Kydd JH, Hussey SB, Mealey RM, Lunn DP. Vaccination of ponies with the IE gene of EHV-1 in a recombinant modified live vaccinia vector protects against clinical and virological disease. *Vet Immunol Immunopathol* 2010; 135:108-17; PMID:20018383; <http://dx.doi.org/10.1016/j.vetimm.2009.11.009>
- [72] Bryant NA, Paillot R, Rash AS, Medcalf E, Montesso F, Ross J, Watson J, Jeggo M, Lewis NS, Newton JR, et al. Comparison of two modern vaccines and previous influenza infection against challenge with an equine influenza virus from the Australian 2007 outbreak. *Vet Res* 2010; 41:19; PMID:19863903; <http://dx.doi.org/10.1051/vetres/2009067>
- [73] Minke JM, Toulemonde CE, Coupier H, Guigal PM, Dinic S, Sindle T, Jessett D, Black L, Bublot M, Pardo MC, et al. Efficacy of a canarypox-vectored recombinant vaccine expressing the hemagglutinin gene of equine influenza H3N8 virus in the protection of ponies from viral challenge. *Am J Vet Res* 2007; 68:213-9; PMID:17269889; <http://dx.doi.org/10.2460/ajvr.68.2.213>
- [74] Paillot R, Kydd JH, Sindle T, Hannant D, Edlund Toulemonde C, Audonnet JC, Minke JM, Daly JM. Antibody and IFN-gamma responses induced by a recombinant canarypox vaccine and challenge infection with equine influenza virus. *Vet Immunol Immunopathol* 2006; 112:225-33; PMID:16621023; <http://dx.doi.org/10.1016/j.vetimm.2006.02.007>
- [75] Edlund Toulemonde C, Daly J, Sindle T, Guigal PM, Audonnet JC, Minke JM. Efficacy of a recombinant equine influenza vaccine against challenge with an American lineage H3N8 influenza virus responsible for the 2003 outbreak in the United Kingdom. *Vet Rec* 2005; 156:367-71; PMID:15816180; <http://dx.doi.org/10.1136/vr.156.12.367>
- [76] Minke JM, Toulemonde CE, Dinic S, Cozette V, Cullinane A, Audonnet JC. Effective priming of foals born to immune dams against influenza by a canarypox-vectored recombinant influenza H3N8 vaccine. *J Comp Pathol* 2007; 137 Suppl 1:S76-80; PMID:17559865; <http://dx.doi.org/10.1016/j.jcpa.2007.04.016>
- [77] Stittelaar KJ, Lacombe V, van Lavieren R, van Amerongen G, Simon J, Cozette V, Swayne DE, Poulet H, Osterhaus AD. Cross-clade immunity in cats vaccinated with a canarypox-vectored avian influenza vaccine. *Vaccine* 2010; 28:4970-6; PMID:20566392; <http://dx.doi.org/10.1016/j.vaccine.2010.05.028>
- [78] Taylor J, Weinberg R, Kawaoka Y, Webster RG, Paoletti E. Protective immunity against avian influenza induced by a fowlpox virus recombinant. *Vaccine* 1988; 6:504-8; PMID:2854339; [http://dx.doi.org/10.1016/0264-410X\(88\)90101-6](http://dx.doi.org/10.1016/0264-410X(88)90101-6)
- [79] Hghihghi HR, Read LR, Mohammadi H, Pei Y, Ursprung C, Nagy E, Behboudi S, Haeryfar SM, Sharif S. Characterization of host responses against a recombinant fowlpox virus-vectored vaccine expressing the hemagglutinin antigen of an avian influenza virus. *Clin Vaccine Immunol* 2010; 17:454-63; PMID:20071494; <http://dx.doi.org/10.1128/CDVI.00487-09>
- [80] Swayne DE, Beck JR, Kinney N. Failure of a recombinant fowl pox-virus vaccine containing an avian influenza hemagglutinin gene to provide consistent protection against influenza in chickens preimmunized with a fowl pox vaccine. *Avian Dis* 2000; 44:132-7; PMID:10737653; <http://dx.doi.org/10.2307/1592516>
- [81] Bublot M, Pritchard N, Cruz JS, Mickle TR, Selleck P, Swayne DE. Efficacy of a fowlpox-vectored avian influenza H5 vaccine against Asian H5N1 highly pathogenic avian influenza virus challenge. *Avian Dis* 2007; 51:498-500; PMID:17494618; <http://dx.doi.org/10.1637/7624-042706R.1>
- [82] Bublot M, Richard-Mazet A, Chanavat-Bizzini S, Le Gros FX, Duboeuf M, Stoll A, Palfi V, Niqueux E, Guionie O, Dren N. Immunogenicity of poxvirus vector avian influenza vaccines in Muscovy and Pekin ducks. *Avian Dis* 2010; 54:232-8; PMID:20521637; <http://dx.doi.org/10.1637/8795-040109-ResNote.1>
- [83] Swayne DE, Garcia M, Beck JR, Kinney N, Suarez DL. Protection against diverse highly pathogenic H5 avian influenza viruses in chickens immunized with a recombinant fowlpox vaccine containing an H5 avian influenza hemagglutinin gene insert. *Vaccine* 2000; 18:1088-95; PMID:10590330; [http://dx.doi.org/10.1016/S0264-410X\(99\)00369-2](http://dx.doi.org/10.1016/S0264-410X(99)00369-2)
- [84] Beard CW, Schnitzlein WM, Tripathy DN. Effect of route of administration on the efficacy of a recombinant fowlpox virus against H5N2 avian influenza. *Avian Dis* 1992; 36:1052-5; PMID:1336657; <http://dx.doi.org/10.2307/1591573>
- [85] Mingxiao M, Ningyi J, Zhenguo W, Ruilin W, Dongliang F, Min Z, Gefen Y, Chang L, Leili J, Kuoshi J, et al. Construction and immunogenicity of recombinant fowlpox vaccines coexpressing HA of AIV H5N1 and chicken IL18. *Vaccine* 2006; 24:4304-11; PMID:16621199; <http://dx.doi.org/10.1016/j.vaccine.2006.03.006>
- [86] Steensels M, Bublot M, Van Borm S, De Vriese J, Lambrecht B, Richard-Mazet A, Chanavat-Bizzini S, Duboeuf M, Le Gros FX, van den Berg T. Prime-boost vaccination with a fowlpox vector and an inactivated avian influenza vaccine is highly immunogenic in Pekin ducks challenged with Asian H5N1 HPAI. *Vaccine* 2009; 27:646-54; PMID:19056442; <http://dx.doi.org/10.1016/j.vaccine.2008.11.044>
- [87] Steensels M, Van Borm S, Lambrecht B, De Vriese J, Le Gros FX, Bublot M, van den Berg T. Efficacy of an inactivated and a fowlpox-vectored vaccine in Muscovy ducks against an Asian H5N1 highly pathogenic avian influenza viral challenge. *Avian Dis* 2007; 51:325-31; PMID:17494576; <http://dx.doi.org/10.1637/7628-042806R.1>

- [88] Bublot M, Manvell RJ, Shell W, Brown IH. High level of protection induced by two fowlpox vector vaccines against a highly pathogenic avian influenza H5N1 challenge in specific-pathogen-free chickens. *Avian Dis* 2010; 54:257-61; PMID:20521642; <http://dx.doi.org/10.1637/8774-033109-ResNote.1>
- [89] Qian C, Chen S, Ding P, Chai M, Xu C, Gan J, Peng D, Liu X. The immune response of a recombinant fowlpox virus coexpressing the HA gene of the H5N1 highly pathogenic avian influenza virus and chicken interleukin 6 gene in ducks. *Vaccine* 2012; 30:6279-86; PMID:22902682; <http://dx.doi.org/10.1016/j.vaccine.2012.08.008>
- [90] Boyle DB, Selleck P, Heine HG. Vaccinating chickens against avian influenza with fowlpox recombinants expressing the H7 haemagglutinin. *Aust Vet J* 2000; 78:44-8; PMID:10736685; <http://dx.doi.org/10.1111/j.1751-0813.2000.tb10359.x>
- [91] Bertran K, Sa ESM, Pantin-Jackwood MJ, Swayne DE. Protection against H7N3 high pathogenicity avian influenza in chickens immunized with a recombinant fowlpox and an inactivated avian influenza vaccines. *Vaccine* 2013; 31:3572-6; PMID:23707445; <http://dx.doi.org/10.1016/j.vaccine.2013.05.039>
- [92] Chen HY, Shang YH, Yao HX, Cui BA, Zhang HY, Wang ZX, Wang YD, Chao AJ, Duan TY. Immune responses of chickens inoculated with a recombinant fowlpox vaccine coexpressing HA of H9N2 avian influenza virus and chicken IL-18. *Antiviral Res* 2011; 91:50-6; PMID:21549153; <http://dx.doi.org/10.1016/j.antiviral.2011.04.007>
- [93] Cheng J, Liu X, Pen D, Liu H. Recombinant fowlpox virus expressing HA from subtype H9N2 of avian influenza virus and its protective immunity against homologous challenge in chickens. *Wei Sheng Wu Xue Bao* 2002; 42:442-7; PMID:12557550
- [94] Webster RG, Kawaoka Y, Taylor J, Weinberg R, Paoletti E. Efficacy of nucleoprotein and haemagglutinin antigens expressed in fowlpox virus as vaccine for influenza in chickens. *Vaccine* 1991; 9:303-8; PMID:1651609; [http://dx.doi.org/10.1016/0264-410X\(91\)90055-B](http://dx.doi.org/10.1016/0264-410X(91)90055-B)
- [95] Qiao C, Jiang Y, Tian G, Wang X, Li C, Xin X, Chen H, Yu K. Recombinant fowlpox virus vector-based vaccine completely protects chickens from H5N1 avian influenza virus. *Antiviral Res* 2009; 81:234-8; PMID:19110002; <http://dx.doi.org/10.1016/j.antiviral.2008.12.002>
- [96] Qiao CL, Yu KZ, Jiang YP, Jia YQ, Tian GB, Liu M, Deng GH, Wang XR, Meng QW, Tang XY. Protection of chickens against highly lethal H5N1 and H7N1 avian influenza viruses with a recombinant fowlpox virus co-expressing H5 haemagglutinin and N1 neuraminidase genes. *Avian Pathol* 2003; 32:25-32; PMID:12745375; <http://dx.doi.org/10.1080/0307945021000070688>
- [97] Karaca K, Swayne DE, Grosenbaugh D, Bublot M, Robles A, Spackman E, Nordgren R. Immunogenicity of fowlpox virus expressing the avian influenza virus H5 gene (TROVAC AIV-H5) in cats. *Clin Diagn Lab Immunol* 2005; 12:1340-2; PMID:16275953
- [98] Rayner JO, Dryga SA, Kamrud KI. Alphavirus vectors and vaccination. *Rev Med Virol* 2002; 12:279-96; PMID:12211042; <http://dx.doi.org/10.1002/rmv.360>
- [99] Berglund P, Fleeton MN, Smerdou C, Liljestrom P. Immunization with recombinant Semliki Forest virus induces protection against influenza challenge in mice. *Vaccine* 1999; 17:497-507; PMID:10073729; [http://dx.doi.org/10.1016/S0264-410X\(98\)00224-2](http://dx.doi.org/10.1016/S0264-410X(98)00224-2)
- [100] Morse MA, Hobeika AC, Osada T, Berglund P, Hubby B, Negri S, Niedzwiecki D, Devi GR, Burnett BK, Clay TM, et al. An alphavirus vector overcomes the presence of neutralizing antibodies and elevated numbers of Tregs to induce immune responses in humans with advanced cancer. *J Clin Invest* 2010; 120:3234-41; PMID:20679728; <http://dx.doi.org/10.1172/JCI42672>
- [101] Pushko P, Parker M, Ludwig GV, Davis NL, Johnston RE, Smith JF. Replicon-helper systems from attenuated Venezuelan equine encephalitis virus: expression of heterologous genes in vitro and immunization against heterologous pathogens in vivo. *Virology* 1997; 239:389-401; PMID:9434729; <http://dx.doi.org/10.1006/viro.1997.8878>
- [102] Uematsu Y, Vajdy M, Lian Y, Perri S, Greer CE, Legg HS, Galli G, Saletti G, Otten GR, Rappuoli R, et al. Lack of interference with immunogenicity of a chimeric alphavirus replicon particle-based influenza vaccine by preexisting antivector immunity. *Clin Vaccine Immunol* 2012; 19:991-8; PMID:22623651; <http://dx.doi.org/10.1128/CVI.00031-12>
- [103] Charles PC, Brown KW, Davis NL, Hart MK, Johnston RE. Mucosal immunity induced by parenteral immunization with a live attenuated Venezuelan equine encephalitis virus vaccine candidate. *Virology* 1997; 228:153-60; <http://dx.doi.org/10.1006/viro.1996.8381>
- [104] Miller A, Center RJ, Stambas J, Deliyannis G, Doherty PC, Howard JL, Turner SJ, Purcell DF. Sindbis virus vectors elicit hemagglutinin-specific humoral and cellular immune responses and offer a dose-sparing strategy for vaccination. *Vaccine* 2008; 26:5641-8; PMID:18761047; <http://dx.doi.org/10.1016/j.vaccine.2008.07.102>
- [105] Tsuji M, Bergmann CC, Takita-Sonoda Y, Murata K, Rodrigues EG, Nussenzweig RS, Zavala F. Recombinant Sindbis viruses expressing a cytotoxic T-lymphocyte epitope of a malaria parasite or of influenza virus elicit protection against the corresponding pathogen in mice. *J Virol* 1998; 72:6907-10; PMID:9658144
- [106] Sheahan T, Whitmore A, Long K, Ferris M, Rockx B, Funkhouser W, Donaldson E, Gralinski L, Collier M, Heise M, et al. Successful vaccination strategies that protect aged mice from lethal challenge from influenza virus and heterologous severe acute respiratory syndrome coronavirus. *J Virol* 2011; 85:217-30; PMID:20980507; <http://dx.doi.org/10.1128/JVI.01805-10>
- [107] Schultz-Cherry S, Dybing JK, Davis NL, Williamson C, Suarez DL, Johnston R, Perdue ML. Influenza virus (A/HK/156/97) hemagglutinin expressed by an alphavirus replicon system protects chickens against lethal infection with Hong Kong-origin H5N1 viruses. *Virology* 2000; 278:55-9; PMID:11112481; <http://dx.doi.org/10.1006/viro.2000.0635>
- [108] Bosworth B, Erdman MM, Stine DL, Harris I, Irwin C, Jens M, Loynachan A, Kamrud K, Harris DL. Replicon particle vaccine protects swine against influenza. *Comp Immunol Microbiol Infect Dis* 2010; 33:e99-e103; PMID:21094422; <http://dx.doi.org/10.1016/j.cimid.2010.05.002>
- [109] Erdman MM, Kamrud KI, Harris DL, Smith J. Alphavirus replicon particle vaccines developed for use in humans induce high levels of antibodies to influenza virus hemagglutinin in swine: proof of concept. *Vaccine* 2010; 28:594-6; PMID:19853679; <http://dx.doi.org/10.1016/j.vaccine.2009.10.015>
- [110] Vander Veen R, Kamrud K, Mogler M, Loynachan AT, McVicker J, Berglund P, Owens G, Timberlake S, Lewis W, Smith J, et al. Rapid development of an efficacious swine vaccine for novel H1N1. *PLoS Curr* 2009; 1:RRN1123; PMID:20029661
- [111] Vander Veen RL, Loynachan AT, Mogler MA, Russell BJ, Harris DL, Kamrud KI. Safety, immunogenicity, and efficacy of an alphavirus replicon-based swine influenza virus hemagglutinin vaccine. *Vaccine* 2012; 30:1944-50; PMID:22269873; <http://dx.doi.org/10.1016/j.vaccine.2012.01.030>
- [112] Vander Veen RL, Mogler MA, Russell BJ, Loynachan AT, Harris DL, Kamrud KI. Haemagglutinin and nucleoprotein replicon particle vaccination of swine protects against the pandemic H1N1 2009 virus. *Vet Rec* 2013; 173:344; PMID:24078226; <http://dx.doi.org/10.1136/vr.101741>
- [113] Bernstein DI, Reap EA, Katen K, Watson A, Smith K, Norberg P, Olmsted RA, Hoepfer A, Morris J, Negri S, et al. Randomized, double-blind, Phase 1 trial of an alphavirus replicon vaccine for cytomegalovirus in CMV seronegative adult volunteers. *Vaccine* 2009; 28:484-93; PMID:19857446; <http://dx.doi.org/10.1016/j.vaccine.2009.09.135>
- [114] Wang J, Osterrieder N. Generation of an infectious clone of duck enteritis virus (DEV) and of a vectored DEV expressing hemagglutinin of H5N1 avian influenza virus. *Virus Res* 2011; 159:23-31; PMID:21549165; <http://dx.doi.org/10.1016/j.virusres.2011.04.013>
- [115] Liu J, Chen P, Jiang Y, Wu L, Zeng X, Tian G, Ge J, Kawaoka Y, Bu Z, Chen H. A duck enteritis virus-vectored bivalent live vaccine provides fast and complete protection against H5N1 avian influenza virus infection in ducks. *J Virol* 2011; 85:10989-98; PMID:21865383; <http://dx.doi.org/10.1128/JVI.05420-11>
- [116] Liu X, Wei S, Liu Y, Fu P, Gao M, Mu X, Liu H, Xing M, Ma B, Wang J. Recombinant duck enteritis virus expressing the HA gene from goose H5 subtype avian influenza virus. *Vaccine* 2013; 31:5953-9; PMID:24144474; <http://dx.doi.org/10.1016/j.vaccine.2013.10.035>

- [117] Zou Z, Hu Y, Liu Z, Zhong W, Cao H, Chen H, Jin M. Efficient strategy for constructing duck enteritis virus-based live attenuated vaccine against homologous and heterologous H5N1 avian influenza virus and duck enteritis virus infection. *Vet Res* 2015; 46:42; PMID:25889564; <http://dx.doi.org/10.1186/s13567-015-0174-3>
- [118] Wang J, Ge A, Xu M, Wang Z, Qiao Y, Gu Y, Liu C, Liu Y, Hou J. Construction of a recombinant duck enteritis virus (DEV) expressing hemagglutinin of H5N1 avian influenza virus based on an infectious clone of DEV vaccine strain and evaluation of its efficacy in ducks and chickens. *Virology* 2015; 12:126; PMID:26263920; <http://dx.doi.org/10.1186/s12985-015-0354-9>
- [119] Luschow D, Werner O, Mettenleiter TC, Fuchs W. Protection of chickens from lethal avian influenza A virus infection by live-virus vaccination with infectious laryngotracheitis virus recombinants expressing the hemagglutinin (H5) gene. *Vaccine* 2001; 19:4249-59; PMID:11457552; [http://dx.doi.org/10.1016/S0264-410X\(01\)00167-0](http://dx.doi.org/10.1016/S0264-410X(01)00167-0)
- [120] Pavlova SP, Veits J, Mettenleiter TC, Fuchs W. Live vaccination with an H5-hemagglutinin-expressing infectious laryngotracheitis virus recombinant protects chickens against different highly pathogenic avian influenza viruses of the H5 subtype. *Vaccine* 2009; 27:5085-90; PMID:19573638; <http://dx.doi.org/10.1016/j.vaccine.2009.06.048>
- [121] Pavlova SP, Veits J, Keil GM, Mettenleiter TC, Fuchs W. Protection of chickens against H5N1 highly pathogenic avian influenza virus infection by live vaccination with infectious laryngotracheitis virus recombinants expressing H5 hemagglutinin and N1 neuraminidase. *Vaccine* 2009; 27:773-85; PMID:19041677; <http://dx.doi.org/10.1016/j.vaccine.2008.11.033>
- [122] Gao H, Cui H, Cui X, Shi X, Zhao Y, Zhao X, Quan Y, Yan S, Zeng W, Wang Y. Expression of HA of HPAI H5N1 virus at US2 gene insertion site of turkey herpesvirus induced better protection than that at US10 gene insertion site. *PLoS One* 2011; 6:e22549; PMID:21818336; <http://dx.doi.org/10.1371/journal.pone.0022549>
- [123] Kapczynski DR, Esaki M, Dorsey KM, Jiang H, Jackwood M, Moraes M, Gardin Y. Vaccine protection of chickens against antigenically diverse H5 highly pathogenic avian influenza isolates with a live HVT vector vaccine expressing the influenza hemagglutinin gene derived from a clade 2.2 avian influenza virus. *Vaccine* 2015; 33:1197-205; PMID:25613723; <http://dx.doi.org/10.1016/j.vaccine.2014.12.028>
- [124] Rauw F, Palya V, Van Borm S, Welby S, Tatar-Kis T, Gardin Y, Dorsey KM, Aly MM, Hassan MK, Soliman MA, et al. Further evidence of antigenic drift and protective efficacy afforded by a recombinant HVT-H5 vaccine against challenge with two antigenically divergent Egyptian clade 2.2.1 HPAI H5N1 strains. *Vaccine* 2011; 29:2590-600; PMID:21292007; <http://dx.doi.org/10.1016/j.vaccine.2011.01.048>
- [125] Li Y, Reddy K, Reid SM, Cox WJ, Brown IH, Britton P, Nair V, Iqbal M. Recombinant herpesvirus of turkeys as a vector-based vaccine against highly pathogenic H7N1 avian influenza and Marek's disease. *Vaccine* 2011; 29:8257-66; PMID:21907750; <http://dx.doi.org/10.1016/j.vaccine.2011.08.115>
- [126] Rauw F, Palya V, Gardin Y, Tatar-Kis T, Dorsey KM, Lambrecht B, van den Berg T. Efficacy of rHVT-AI vector vaccine in broilers with passive immunity against challenge with two antigenically divergent Egyptian clade 2.2.1 HPAI H5N1 strains. *Avian Dis* 2012; 56:913-22; PMID:23402112; <http://dx.doi.org/10.1637/10172-041012-Reg.1>
- [127] Cui H, Gao H, Cui X, Zhao Y, Shi X, Li Q, Yan S, Gao M, Wang M, Liu C, et al. Avirulent Marek's disease virus type 1 strain 814 vectored vaccine expressing avian influenza (AI) virus H5 haemagglutinin induced better protection than turkey herpesvirus vectored AI vaccine. *PLoS One* 2013; 8:e53340; PMID:23301062; <http://dx.doi.org/10.1371/journal.pone.0053340>
- [128] Pomeranz LE, Reynolds AE, Hengartner CJ. Molecular biology of pseudorabies virus: impact on neurovirology and veterinary medicine. *Microbiol Mol Biol Rev* 2005; 69:462-500; PMID:16148307; <http://dx.doi.org/10.1128/MMBR.69.3.462-500.2005>
- [129] Klingbeil K, Lange E, Blohm U, Teifke JP, Mettenleiter TC, Fuchs W. Protection of pigs against pandemic swine origin H1N1 influenza A virus infection by hemagglutinin- or neuraminidase-expressing attenuated pseudorabies virus recombinants. *Virus Res* 2015; 199:20-30; PMID:25599604; <http://dx.doi.org/10.1016/j.virusres.2015.01.009>
- [130] Klingbeil K, Lange E, Teifke JP, Mettenleiter TC, Fuchs W. Immunization of pigs with an attenuated pseudorabies virus recombinant expressing the haemagglutinin of pandemic swine origin H1N1 influenza A virus. *J Gen Virol* 2014; 95:948-59; PMID:24431235; <http://dx.doi.org/10.1099/vir.0.059253-0>
- [131] Tian ZJ, Zhou GH, Zheng BL, Qiu HJ, Ni JQ, Yang HL, Yin XN, Hu SP, Tong GZ. A recombinant pseudorabies virus encoding the HA gene from H3N2 subtype swine influenza virus protects mice from virulent challenge. *Vet Immunol Immunopathol* 2006; 111:211-8; PMID:16621018; <http://dx.doi.org/10.1016/j.vetimm.2006.01.015>
- [132] Rosas C, Van de Walle GR, Metzger SM, Hoelzer K, Dubovi EJ, Kim SG, Parrish CR, Osterrieder N. Evaluation of a vectored equine herpesvirus type 1 (EHV-1) vaccine expressing H3 haemagglutinin in the protection of dogs against canine influenza. *Vaccine* 2008; 26:2335-43; PMID:18407383; <http://dx.doi.org/10.1016/j.vaccine.2008.02.064>
- [133] Van de Walle GR, May MA, Peters ST, Metzger SM, Rosas CT, Osterrieder N. A vectored equine herpesvirus type 1 (EHV-1) vaccine elicits protective immune responses against EHV-1 and H3N8 equine influenza virus. *Vaccine* 2010; 28:1048-55; PMID:19897066; <http://dx.doi.org/10.1016/j.vaccine.2009.10.123>
- [134] Said A, Damiani A, Ma G, Kalthoff D, Beer M, Osterrieder N. An equine herpesvirus 1 (EHV-1) vectored H1 vaccine protects against challenge with swine-origin influenza virus H1N1. *Vet Microbiol* 2011; 154:113-23; PMID:21803510; <http://dx.doi.org/10.1016/j.vetmic.2011.07.003>
- [135] Said A, Lange E, Beer M, Damiani A, Osterrieder N. Recombinant equine herpesvirus 1 (EHV-1) vaccine protects pigs against challenge with influenza A(H1N1)pmd09. *Virus Res* 2013; 173:371-6; PMID:23333290; <http://dx.doi.org/10.1016/j.virusres.2013.01.004>
- [136] Roberts A, Buonocore L, Price R, Forman J, Rose JK. Attenuated vesicular stomatitis viruses as vaccine vectors. *J Virol* 1999; 73:3723-32; PMID:10196265
- [137] Roberts A, Kretzschmar E, Perkins AS, Forman J, Price R, Buonocore L, Kawaoaka Y, Rose JK. Vaccination with a recombinant vesicular stomatitis virus expressing an influenza virus hemagglutinin provides complete protection from influenza virus challenge. *J Virol* 1998; 72:4704-11; PMID:9573234
- [138] Kopecky-Bromberg SA, Palese P. Recombinant vectors as influenza vaccines. *Curr Top Microbiol Immunol* 2009; 333:243-67; PMID:19768410
- [139] Sabin AB, Olitsky PK. Influence of Host Factors on neuroinvasiveness of vesicular stomatitis virus : I. Effect of age on the invasion of the brain by virus instilled in the nose. *J Exp Med* 1937; 66:15-34; PMID:19870647; <http://dx.doi.org/10.1084/jem.66.1.15>
- [140] Barefoot BE, Sample CJ, Ramsburg EA. Recombinant vesicular stomatitis virus expressing influenza nucleoprotein induces CD8 T-cell responses that enhance antibody-mediated protection after lethal challenge with influenza virus. *Clin Vaccine Immunol* 2009; 16:488-98; PMID:19244472; <http://dx.doi.org/10.1128/CVI.00451-08>
- [141] Ryder AB, Nachbagauer R, Buonocore L, Palese P, Krammer F, Rose JK. Vaccination with vesicular stomatitis Virus-Vectored Chimeric Hemagglutinins protects mice against divergent influenza virus challenge strains. *J Virol* 2015; 90:2544-50; PMID:26676789; <http://dx.doi.org/10.1128/JVI.02598-15>
- [142] Kalhor NH, Veits J, Rautenschlein S, Zimmer G. A recombinant vesicular stomatitis virus replicon vaccine protects chickens from highly pathogenic avian influenza virus (H7N1). *Vaccine* 2009; 27:1174-83; PMID:19135116; <http://dx.doi.org/10.1016/j.vaccine.2008.12.019>
- [143] Halbherr SJ, Brostoff T, Tippenhauer M, Locher S, Berger Rentsch M, Zimmer G. Vaccination with recombinant RNA replicon particles protects chickens from H5N1 highly pathogenic avian influenza virus. *PLoS One* 2013; 8:e66059; PMID:23762463; <http://dx.doi.org/10.1371/journal.pone.0066059>
- [144] Schwartz JA, Buonocore L, Roberts A, Suguitan A, Jr, Kobasa D, Kobinger G, Feldmann H, Subbarao K, Rose JK. Vesicular stomatitis virus vectors expressing avian influenza H5 HA induce cross-neutralizing antibodies and long-term protection. *Virology* 2007; 366:166-73; PMID:17524441; <http://dx.doi.org/10.1016/j.viro.2007.04.021>
- [145] Schwartz JA, Buonocore L, Suguitan A, Jr, Hunter M, Marx PA, Subbarao K, Rose JK. Vesicular stomatitis virus-based H5N1 avian

- influenza vaccines induce potent cross-clade neutralizing antibodies in rhesus macaques. *J Virol* 2011; 85:4602-5; PMID:21325423; <http://dx.doi.org/10.1128/JVI.02491-10>
- [146] Schwartz JA, Buonocore L, Suguitan AL, Jr, Silaghi A, Kobasa D, Kobinger G, Feldmann H, Subbarao K, Rose JK. Potent vesicular stomatitis virus-based avian influenza vaccines provide long-term sterilizing immunity against heterologous challenge. *J Virol* 2010; 84:4611-8; PMID:20181720; <http://dx.doi.org/10.1128/JVI.02637-09>
- [147] Krammer F, Palese P. Influenza virus hemagglutinin stalk-based antibodies and vaccines. *Curr Opin Virol* 2013; 3:521-30; PMID:23978327; <http://dx.doi.org/10.1016/j.coviro.2013.07.007>
- [148] Nakaya T, Cros J, Park MS, Nakaya Y, Zheng H, Sagrera A, Villar E, Garcia-Sastre A, Palese P. Recombinant Newcastle disease virus as a vaccine vector. *J Virol* 2001; 75:11868-73; PMID:11689668; <http://dx.doi.org/10.1128/JVI.75.23.11868-11873.2001>
- [149] Ferreira HL, Pirlot JF, Reynard F, van den Berg T, Bublot M, Lambrecht B. Immune responses and protection against H5N1 highly pathogenic avian influenza virus induced by the Newcastle disease virus H5 vaccine in ducks. *Avian Dis* 2012; 56:940-8; PMID:23402116; <http://dx.doi.org/10.1637/10148-040812-ResNote.1>
- [150] Ferreira HL, Rauw F, Pirlot JF, Reynard F, van den Berg T, Bublot M, Lambrecht B. Comparison of single 1-day-old chick vaccination using a Newcastle disease virus vector with a prime/boost vaccination scheme against a highly pathogenic avian influenza H5N1 challenge. *Avian Pathol* 2014; 43:68-77; PMID:24320551; <http://dx.doi.org/10.1080/03079457.2013.873111>
- [151] Kim SH, Paldurai A, Xiao S, Collins PL, Samal SK. Modified Newcastle disease virus vectors expressing the H5 hemagglutinin induce enhanced protection against highly pathogenic H5N1 avian influenza virus in chickens. *Vaccine* 2014; 32:4428-35; PMID:24968158; <http://dx.doi.org/10.1016/j.vaccine.2014.06.061>
- [152] Lardinois A, Steensels M, Lambrecht B, Desloges N, Rahaus M, Rebeski D, van den Berg T. Potency of a recombinant NDV-H5 vaccine against various HPAI H5N1 virus challenges in SPF chickens. *Avian Dis* 2012; 56:928-36; PMID:23402114; <http://dx.doi.org/10.1637/10173-041012-ResNote.1>
- [153] Lozano-Dubernard B, Soto-Priante E, Sarfati-Mizrahi D, Castro-Peralta F, Flores-Castro R, Loza-Rubio E, Gay-Gutierrez M. Protection and differentiation of infected from vaccinated animals by an inactivated recombinant Newcastle disease virus/avian influenza H5 vaccine. *Avian Dis* 2010; 54:242-5; PMID:20521639; <http://dx.doi.org/10.1637/8767-033109-ResNote.1>
- [154] Nayak B, Rout SN, Kumar S, Khalil MS, Fouda MM, Ahmed LE, Earhart KC, Perez DR, Collins PL, Samal SK. Immunization of chickens with Newcastle disease virus expressing H5 hemagglutinin protects against highly pathogenic H5N1 avian influenza viruses. *PLoS One* 2009; 4:e6509; PMID:19654873; <http://dx.doi.org/10.1371/journal.pone.0006509>
- [155] Niqueux E, Guionie O, Amelot M, Jestin V. Prime-boost vaccination with recombinant H5-fowlpox and Newcastle disease virus vectors affords lasting protection in SPF Muscovy ducks against highly pathogenic H5N1 influenza virus. *Vaccine* 2013; 31:4121-8; PMID:23845804; <http://dx.doi.org/10.1016/j.vaccine.2013.06.074>
- [156] Ramp K, Veits J, Deckers D, Rudolf M, Grund C, Mettenleiter TC, Romer-Oberdorfer A. Coexpression of avian influenza virus H5 and N1 by recombinant Newcastle disease virus and the impact on immune response in chickens. *Avian Dis* 2011; 55:413-21; PMID:22017039; <http://dx.doi.org/10.1637/9652-011111-Reg.1>
- [157] Romer-Oberdorfer A, Veits J, Helferich D, Mettenleiter TC. Level of protection of chickens against highly pathogenic H5 avian influenza virus with Newcastle disease virus based live attenuated vector vaccine depends on homology of H5 sequence between vaccine and challenge virus. *Vaccine* 2008; 26:2307-13; PMID:18395947; <http://dx.doi.org/10.1016/j.vaccine.2008.02.061>
- [158] Veits J, Wiesner D, Fuchs W, Hoffmann B, Granzow H, Starick E, Mundt E, Schirmer H, Mebatsion T, Mettenleiter TC, et al. Newcastle disease virus expressing H5 hemagglutinin gene protects chickens against Newcastle disease and avian influenza. *Proc Natl Acad Sci U S A* 2006; 103:8197-202; PMID:16717197; <http://dx.doi.org/10.1073/pnas.0602461103>
- [159] Cornelissen LA, de Leeuw OS, Tacke MG, Klos HC, de Vries RP, de Boer-Luijze EA, van Zoelen-Bos DJ, Rigter A, Rottier PJ, Moormann RJ, et al. Protective efficacy of Newcastle disease virus expressing soluble trimeric hemagglutinin against highly pathogenic H5N1 influenza in chickens and mice. *PLoS One* 2012; 7:e44447; PMID:22952980; <http://dx.doi.org/10.1371/journal.pone.0044447>
- [160] Ge J, Deng G, Wen Z, Tian G, Wang Y, Shi J, Wang X, Li Y, Hu S, Jiang Y, et al. Newcastle disease virus-based live attenuated vaccine completely protects chickens and mice from lethal challenge of homologous and heterologous H5N1 avian influenza viruses. *J Virol* 2007; 81:150-8; PMID:17050610; <http://dx.doi.org/10.1128/JVI.01514-06>
- [161] Liu Q, Mena I, Ma J, Bawa B, Krammer F, Lyoo YS, Lang Y, Morozov I, Mahardika GN, Ma W, et al. Newcastle disease Virus-Vectored H7 and H5 live vaccines protect chickens from challenge with H7N9 or H5N1 avian influenza viruses. *J Virol* 2015; 89:7401-8; PMID:25926639; <http://dx.doi.org/10.1128/JVI.00031-15>
- [162] Sarfati-Mizrahi D, Lozano-Dubernard B, Soto-Priante E, Castro-Peralta F, Flores-Castro R, Loza-Rubio E, Gay-Gutierrez M. Protective dose of a recombinant Newcastle disease LaSota-avian influenza virus H5 vaccine against H5N2 highly pathogenic avian influenza virus and velogenic viscerotropic Newcastle disease virus in broilers with high maternal antibody levels. *Avian Dis* 2010; 54:239-41; PMID:20521638; <http://dx.doi.org/10.1637/8735-032509-Reg.1>
- [163] Steglich C, Grund C, Ramp K, Breithaupt A, Hoper D, Keil G, Veits J, Ziller M, Granzow H, Mettenleiter TC, et al. Chimeric newcastle disease virus protects chickens against avian influenza in the presence of maternally derived NDV immunity. *PLoS One* 2013; 8:e72530; PMID:24023747; <http://dx.doi.org/10.1371/journal.pone.0072530>
- [164] Ge J, Tian G, Zeng X, Jiang Y, Chen H, Bua Z. Generation and evaluation of a Newcastle disease virus-based H9 avian influenza live vaccine. *Avian Dis* 2010; 54:294-6; PMID:20521648; <http://dx.doi.org/10.1637/8731-032509-ResNote.1>
- [165] Schroer D, Veits J, Grund C, Dauber M, Keil G, Granzow H, Mettenleiter TC, Romer-Oberdorfer A. Vaccination with Newcastle disease virus vectored vaccine protects chickens against highly pathogenic H7 avian influenza virus. *Avian Dis* 2009; 53:190-7; PMID:19630223; <http://dx.doi.org/10.1637/8416-072308-Reg.1>
- [166] Schroer D, Veits J, Keil G, Romer-Oberdorfer A, Weber S, Mettenleiter TC. Efficacy of Newcastle disease virus recombinant expressing avian influenza virus H6 hemagglutinin against Newcastle disease and low pathogenic avian influenza in chickens and turkeys. *Avian Dis* 2011; 55:201-11; PMID:21793434; <http://dx.doi.org/10.1637/9539-092710-Reg.1>
- [167] Swaine DE, Suarez DL, Schultz-Cherry S, Tumpey TM, King DJ, Nakaya T, Palese P, Garcia-Sastre A. Recombinant paramyxovirus type 1-avian influenza-H7 virus as a vaccine for protection of chickens against influenza and Newcastle disease. *Avian Dis* 2003; 47:1047-50; PMID:14575108; <http://dx.doi.org/10.1637/0005-2086-47.s3.1047>
- [168] Yang S, Niu S, Guo Z, Yuan Y, Xue K, Liu S, Jin H. Cross-protective immunity against influenza A/H1N1 virus challenge in mice immunized with recombinant vaccine expressing HA gene of influenza A/H5N1 virus. *Virol J* 2013; 10:291; PMID:24053449; <http://dx.doi.org/10.1186/1743-422X-10-291>
- [169] DiNapoli JM, Nayak B, Yang L, Finneyfrock BW, Cook A, Andersen H, Torres-Velez F, Murphy BR, Samal SK, Collins PL, et al. Newcastle disease virus-vectored vaccines expressing the hemagglutinin or neuraminidase protein of H5N1 highly pathogenic avian influenza virus protect against virus challenge in monkeys. *J Virol* 2010; 84:1489-503; PMID:19923177; <http://dx.doi.org/10.1128/JVI.01946-09>
- [170] DiNapoli JM, Yang L, Suguitan A, Jr, Elankumaran S, Dorward DW, Murphy BR, Samal SK, Collins PL, Bukreyev A. Immunization of primates with a Newcastle disease virus-vectored vaccine via the respiratory tract induces a high titer of serum neutralizing antibodies against highly pathogenic avian influenza virus. *J Virol* 2007; 81:11560-8; PMID:17715243 <http://dx.doi.org/10.1128/JVI.00713-07>
- [171] Kost TA, Condreay JP. Recombinant baculoviruses as mammalian cell gene-delivery vectors. *Trends Biotechnol* 2002; 20:173-80; PMID:11906750; [http://dx.doi.org/10.1016/S0167-7799\(01\)01911-4](http://dx.doi.org/10.1016/S0167-7799(01)01911-4)

- [172] Abe T, Takahashi H, Hamazaki H, Miyano-Kurosaki N, Matsuura Y, Takaku H. Baculovirus induces an innate immune response and confers protection from lethal influenza virus infection in mice. *J Immunol* 2003; 171:1133-9; PMID:12874198; <http://dx.doi.org/10.4049/jimmunol.171.3.1133>
- [173] Prabakaran M, Madhan S, Prabhu N, Qiang J, Kwang J. Gastrointestinal delivery of baculovirus displaying influenza virus hemagglutinin protects mice against heterologous H5N1 infection. *J Virol* 2010; 84:3201-9; PMID:20071572; <http://dx.doi.org/10.1128/JVI.02175-09>
- [174] Wu Q, Fang L, Wu X, Li B, Luo R, Yu Z, Jin M, Chen H, Xiao S. A pseudotype baculovirus-mediated vaccine confers protective immunity against lethal challenge with H5N1 avian influenza virus in mice and chickens. *Mol Immunol* 2009; 46:2210-7; PMID:19446339; <http://dx.doi.org/10.1016/j.molimm.2009.04.017>
- [175] Prabakaran M, Kolpe AB, He F, Kwang J. Cross-protective efficacy of bivalent recombinant baculoviral vaccine against heterologous influenza H5N1 challenge. *Vaccine* 2013; 31:1385-92; PMID:23328313; <http://dx.doi.org/10.1016/j.vaccine.2013.01.003>
- [176] Wu Q, Xiao S, Fan H, Li Y, Xu J, Li Z, Lu W, Su X, Zou W, Jin M, et al. Protective immunity elicited by a pseudotyped baculovirus-mediated bivalent H5N1 influenza vaccine. *Antiviral Res* 2011; 92:493-6; PMID:22020305; <http://dx.doi.org/10.1016/j.antiviral.2011.10.001>
- [177] Lin W, Fan H, Cheng X, Ye Y, Chen X, Ren T, Qi W, Liao M. A baculovirus dual expression system-based vaccine confers complete protection against lethal challenge with H9N2 avian influenza virus in mice. *Virol J* 2011; 8:273; PMID:21639929; <http://dx.doi.org/10.1186/1743-422X-8-273>
- [178] Musthaq SK, Kumar SR, Szyporta M, Kwang J. Immunization with baculovirus displayed H6 hemagglutinin vaccine protects mice against lethal H6 influenza virus challenge. *Antiviral Res* 2014; 109:42-53; PMID:24973759; <http://dx.doi.org/10.1016/j.antiviral.2014.06.002>
- [179] Prabakaran M, Kumar SR, Raj KV, Wu X, He F, Zhou J, Kwang J. Cross-protective efficacy of baculovirus displayed hemagglutinin against highly pathogenic influenza H7 subtypes. *Antiviral Res* 2014; 109:149-59; PMID:24997413; <http://dx.doi.org/10.1016/j.antiviral.2014.06.017>
- [180] Chen Z, Xu P, Salyards GW, Harvey SB, Rada B, Fu ZF, He B. Evaluating a parainfluenza virus 5-based vaccine in a host with pre-existing immunity against parainfluenza virus 5. *PLoS One* 2012; 7:e50144; PMID:23185558; <http://dx.doi.org/10.1371/journal.pone.0050144>
- [181] Tompkins SM, Lin Y, Leser GP, Kramer KA, Haas DL, Howerth EW, Xu J, Kennett MJ, Durbin RK, Durbin JE, et al. Recombinant parainfluenza virus 5 (PIV5) expressing the influenza A virus hemagglutinin provides immunity in mice to influenza A virus challenge. *Virology* 2007; 362:139-50; PMID:17254623; <http://dx.doi.org/10.1016/j.virol.2006.12.005>
- [182] Li Z, Gabbard JD, Johnson S, Dlugolenski D, Phan S, Tompkins SM, He B. Efficacy of a parainfluenza virus 5 (PIV5)-based H7N9 vaccine in mice and guinea pigs: antibody titer towards HA was not a good indicator for protection. *PLoS One* 2015; 10:e0120355; PMID:25803697; <http://dx.doi.org/10.1371/journal.pone.0120355>
- [183] Li Z, Gabbard JD, Mooney A, Gao X, Chen Z, Place RJ, Tompkins SM, He B. Single-dose vaccination of a recombinant parainfluenza virus 5 expressing NP from H5N1 virus provides broad immunity against influenza A viruses. *J Virol* 2013; 87:5985-93; PMID:23514880; <http://dx.doi.org/10.1128/JVI.00120-13>
- [184] Zhang J. Advances and future challenges in recombinant adenoviral vectored H5N1 influenza vaccines. *Viruses* 2012; 4:2711-35; PMID:23202501; <http://dx.doi.org/10.3390/v4112711>
- [185] Gaydos CA, Gaydos JC. Adenovirus vaccines in the US military. *Mil Med* 1995; 160:300-4; PMID:7659229
- [186] Marshall E. Gene therapy death prompts review of adenovirus vector. *Science* 1999; 286:2244-5; PMID:10636774; <http://dx.doi.org/10.1126/science.286.5448.2244>
- [187] Duerr A, Huang Y, Buchbinder S, Coombs RW, Sanchez J, del Rio C, Casapia M, Santiago S, Gilbert P, Corey L, et al. Extended follow-up confirms early vaccine-enhanced risk of HIV acquisition and demonstrates waning effect over time among participants in a randomized trial of recombinant adenovirus HIV vaccine (Step Study). *J Infect Dis* 2012; 206:258-66; PMID:22561365; <http://dx.doi.org/10.1093/infdis/jis342>
- [188] Van Kampen KR, Shi Z, Gao P, Zhang J, Foster KW, Chen DT, Marks D, Elmets CA, Tang DC. Safety and immunogenicity of adenovirus-vectored nasal and epicutaneous influenza vaccines in humans. *Vaccine* 2005; 23:1029-36; PMID:15620476; <http://dx.doi.org/10.1016/j.vaccine.2004.07.043>
- [189] Gurwith M, Lock M, Taylor EM, Ishioka G, Alexander J, Mayall T, Ervin JE, Greenberg RN, Strout C, Treanor JJ, et al. Safety and immunogenicity of an oral, replicating adenovirus serotype 4 vector vaccine for H5N1 influenza: a randomised, double-blind, placebo-controlled, phase 1 study. *Lancet Infect Dis* 2013; 13:238-50; PMID:23369412; [http://dx.doi.org/10.1016/S1473-3099\(12\)70345-6](http://dx.doi.org/10.1016/S1473-3099(12)70345-6)
- [190] Antrobus RD, Coughlan L, Berthoud TK, Dicks MD, Hill AV, Lambe T, Gilbert SC. Clinical assessment of a novel recombinant simian adenovirus ChAdOx1 as a vectored vaccine expressing conserved influenza A antigens. *Mol Ther* 2014; 22:668-74; PMID:24374965; <http://dx.doi.org/10.1038/mt.2013.284>
- [191] Liebowitz D, Lindbloom JD, Brandl JR, Garg SJ, Tucker SN. High titre neutralising antibodies to influenza after oral tablet immunisation: a phase 1, randomised, placebo-controlled trial. *Lancet Infect Dis* 2015; 15:1041-8; PMID:26333337; [http://dx.doi.org/10.1016/S1473-3099\(15\)00266-2](http://dx.doi.org/10.1016/S1473-3099(15)00266-2)
- [192] Patel A, Tikoo S, Kobinger G. A porcine adenovirus with low human seroprevalence is a promising alternative vaccine vector to human adenovirus 5 in an H5N1 virus disease model. *PLoS One* 2010; 5:e15301; PMID:21179494; <http://dx.doi.org/10.1371/journal.pone.0015301>
- [193] Roy S, Kobinger GP, Lin J, Figueredo J, Calcedo R, Kobasa D, Wilson JM. Partial protection against H5N1 influenza in mice with a single dose of a chimpanzee adenovirus vector expressing nucleoprotein. *Vaccine* 2007; 25:6845-51; PMID:17728024; <http://dx.doi.org/10.1016/j.vaccine.2007.07.035>
- [194] Singh N, Pandey A, Jayashankar L, Mittal SK. Bovine adenoviral vector-based H5N1 influenza vaccine overcomes exceptionally high levels of pre-existing immunity against human adenovirus. *Mol Ther* 2008; 16:965-71; PMID:18301400; <http://dx.doi.org/10.1038/mt.2008.12>
- [195] Weaver EA, Barry MA. Low seroprevalent species D adenovirus vectors as influenza vaccines. *PLoS One* 2013; 8:e73313; PMID:23991187; <http://dx.doi.org/10.1371/journal.pone.0073313>
- [196] Tang M, Harp JA, Wesley RD. Recombinant adenovirus encoding the HA gene from swine H3N2 influenza virus partially protects mice from challenge with heterologous virus: A/HK/1/68 (H3N2). *Arch Virol* 2002; 147:2125-41; PMID:12417948; <http://dx.doi.org/10.1007/s00705-002-0870-y>
- [197] Wesley RD, Tang M, Lager KM. Protection of weaned pigs by vaccination with human adenovirus 5 recombinant viruses expressing the hemagglutinin and the nucleoprotein of H3N2 swine influenza virus. *Vaccine* 2004; 22:3427-34; PMID:15308368; <http://dx.doi.org/10.1016/j.vaccine.2004.02.040>
- [198] Wesley RD, Lager KM. Overcoming maternal antibody interference by vaccination with human adenovirus 5 recombinant viruses expressing the hemagglutinin and the nucleoprotein of swine influenza virus. *Vet Microbiol* 2006; 118:67-75; PMID:16939702; <http://dx.doi.org/10.1016/j.vetmic.2006.07.014>
- [199] Kim JY, Choi Y, Nguyen HH, Song MK, Chang J. Mucosal immunization with recombinant adenovirus encoding soluble globular head of hemagglutinin protects mice against lethal influenza virus infection. *Immune Netw* 2013; 13:275-82; PMID:24385946; <http://dx.doi.org/10.4110/in.2013.13.6.275>
- [200] Wei CJ, Boyington JC, McTamney PM, Kong WP, Pearce MB, Xu L, Andersen H, Rao S, Tumpey TM, Yang ZY, et al. Induction of broadly neutralizing H1N1 influenza antibodies by vaccination. *Science* 2010; 329:1060-4; PMID:20647428; <http://dx.doi.org/10.1126/science.1192517>
- [201] Braucher DR, Henningson JN, Loving CL, Vincent AL, Kim E, Steitz J, Gambotto AA, Kehrl ME, Jr. Intranasal vaccination with replication-defective adenovirus type 5 encoding influenza virus hemagglutinin elicits protective immunity to homologous challenge and partial protection to heterologous challenge in pigs. *Clin*

- Vaccine Immunol 2012; 19:1722-9; PMID:22933397; <http://dx.doi.org/10.1128/CVI.00315-12>
- [202] Alexander J, Ward S, Mendy J, Manayani DJ, Farness P, Avanzini JB, Guenther B, Garduno F, Jow L, Snarsky V, et al. Pre-clinical evaluation of a replication-competent recombinant adenovirus serotype 4 vaccine expressing influenza H5 hemagglutinin. *PLoS One* 2012; 7:e31177; PMID:22363572; <http://dx.doi.org/10.1371/journal.pone.0031177>
- [203] Gao W, Soloff AC, Lu X, Montecalvo A, Nguyen DC, Matsuoka Y, Robbins PD, Swayne DE, Donis RO, Katz JM, et al. Protection of mice and poultry from lethal H5N1 avian influenza virus through adenovirus-based immunization. *J Virol* 2006; 80:1959-64; PMID:16439551; <http://dx.doi.org/10.1128/JVI.80.4.1959-1964.2006>
- [204] Scallan CD, Tingley DW, Lindbloom JD, Toomey JS, Tucker SN. An adenovirus-based vaccine with a double-stranded RNA adjuvant protects mice and ferrets against H5N1 avian influenza in oral delivery models. *Clin Vaccine Immunol* 2013; 20:85-94; PMID:23155123; <http://dx.doi.org/10.1128/CVI.00552-12>
- [205] Hoelscher MA, Garg S, Bangari DS, Belsler JA, Lu X, Stephenson I, Bright RA, Katz JM, Mittal SK, Sambhara S. Development of adenoviral-vector-based pandemic influenza vaccine against antigenically distinct human H5N1 strains in mice. *Lancet* 2006; 367:475-81; PMID:16473124; [http://dx.doi.org/10.1016/S0140-6736\(06\)68076-8](http://dx.doi.org/10.1016/S0140-6736(06)68076-8)
- [206] Toro H, Tang DC, Suarez DL, Zhang J, Shi Z. Protection of chickens against avian influenza with non-replicating adenovirus-vectored vaccine. *Vaccine* 2008; 26:2640-6; PMID:18384919; <http://dx.doi.org/10.1016/j.vaccine.2008.02.056>
- [207] Vemula SV, Ahi YS, Swaim AM, Katz JM, Donis R, Sambhara S, Mittal SK. Broadly protective adenovirus-based multivalent vaccines against highly pathogenic avian influenza viruses for pandemic preparedness. *PLoS One* 2013; 8:e62496; PMID:23638099; <http://dx.doi.org/10.1371/journal.pone.0062496>
- [208] Lin SC, Liu WC, Jan JT, Wu SC. Glycan masking of hemagglutinin for adenovirus vector and recombinant protein immunizations elicits broadly neutralizing antibodies against H5N1 avian influenza viruses. *PLoS One* 2014; 9:e92822; PMID:24671139; <http://dx.doi.org/10.1371/journal.pone.0092822>
- [209] Hashem A, Jaentschke B, Gravel C, Tocchi M, Doyle T, Rosu-Myles M, He R, Li X. Subcutaneous immunization with recombinant adenovirus expressing influenza A nucleoprotein protects mice against lethal viral challenge. *Hum Vaccin Immunother* 2012; 8:425-30; PMID:22370512; <http://dx.doi.org/10.4161/hv.19109>
- [210] Soboleski MR, Gabbard JD, Price GE, Mispion JA, Lo CY, Perez DR, Ye J, Tompkins SM, Epstein SL. Cold-adapted influenza and recombinant adenovirus vaccines induce cross-protective immunity against pH1N1 challenge in mice. *PLoS One* 2011; 6:e21937; PMID:21789196; <http://dx.doi.org/10.1371/journal.pone.0021937>
- [211] Price GE, Lo CY, Mispion JA, Epstein SL. Mucosal immunization with a candidate universal influenza vaccine reduces virus transmission in a mouse model. *J Virol* 2014; 88:6019-30; PMID:24623430; <http://dx.doi.org/10.1128/JVI.03101-13>
- [212] Zhang H, Tang X, Zhu C, Song Y, Yin J, Xu J, Ertl HC, Zhou D. Adenovirus-mediated artificial MicroRNAs targeting matrix or nucleoprotein genes protect mice against lethal influenza virus challenge. *Gene Ther* 2015; 22:653-62; PMID:25835311; <http://dx.doi.org/10.1038/gt.2015.31>
- [213] Jones FR, Gabitzsch ES, Xu Y, Balint JP, Borisevich V, Smith J, Smith J, Peng BH, Walker A, Salazar M, et al. Prevention of influenza virus shedding and protection from lethal H1N1 challenge using a consensus 2009 H1N1 HA and NA adenovirus vector vaccine. *Vaccine* 2011; 29:7020-6; PMID:21821082; <http://dx.doi.org/10.1016/j.vaccine.2011.07.073>
- [214] Holman DH, Wang D, Raja NU, Luo M, Moore KM, Woraratanad-harm J, Mytle N, Dong JY. Multi-antigen vaccines based on complex adenovirus vectors induce protective immune responses against H5N1 avian influenza viruses. *Vaccine* 2008; 26:2627-39; PMID:18395306; <http://dx.doi.org/10.1016/j.vaccine.2008.02.053>
- [215] Epstein SL, Kong WP, Mispion JA, Lo CY, Tumpey TM, Xu L, Nabel GJ. Protection against multiple influenza A subtypes by vaccination with highly conserved nucleoprotein. *Vaccine* 2005; 23:5404-10; PMID:16011865; <http://dx.doi.org/10.1016/j.vaccine.2005.04.047>
- [216] Rao SS, Kong WP, Wei CJ, Van Hoeven N, Gorres JP, Nason M, Andersen H, Tumpey TM, Nabel GJ. Comparative efficacy of hemagglutinin, nucleoprotein, and matrix 2 protein gene-based vaccination against H5N1 influenza in mouse and ferret. *PLoS One* 2010; 5:e9812; PMID:20352112; <http://dx.doi.org/10.1371/journal.pone.0009812>
- [217] Price GE, Soboleski MR, Lo CY, Mispion JA, Pappas C, Houser KV, Tumpey TM, Epstein SL. Vaccination focusing immunity on conserved antigens protects mice and ferrets against virulent H1N1 and H5N1 influenza A viruses. *Vaccine* 2009; 27:6512-21; PMID:19729082; <http://dx.doi.org/10.1016/j.vaccine.2009.08.053>
- [218] Lai CM, Lai YK, Rakoczy PE. Adenovirus and adeno-associated virus vectors. *DNA Cell Biol* 2002; 21:895-913; PMID:12573049; <http://dx.doi.org/10.1089/104454902762053855>
- [219] Manning WC, Paliard X, Zhou S, Pat Bland M, Lee AY, Hong K, Walker CM, Escobedo JA, Dworki V. Genetic immunization with adeno-associated virus vectors expressing herpes simplex virus type 2 glycoproteins B and D. *J Virol* 1997; 71:7960-2; PMID:9311887
- [220] Nieto K, Salvetti A. AAV vectors vaccines against infectious diseases. *Front Immunol* 2014; 5:5; PMID:24478774; <http://dx.doi.org/10.3389/fimmu.2014.00005>
- [221] Lin J, Calcedo R, Vandenberghe LH, Bell P, Somanathan S, Wilson JM. A new genetic vaccine platform based on an adeno-associated virus isolated from a rhesus macaque. *J Virol* 2009; 83:12738-50; PMID:19812149; <http://dx.doi.org/10.1128/JVI.01441-09>
- [222] Xin KQ, Urabe M, Yang J, Nomiya K, Mizukami H, Hamajima K, Nomiya H, Saito T, Imai M, Monahan J, et al. A novel recombinant adeno-associated virus vaccine induces a long-term humoral immune response to human immunodeficiency virus. *Hum Gene Ther* 2001; 12:1047-61; PMID:11399227; <http://dx.doi.org/10.1089/104303401750214276>
- [223] Sipo I, Knauf M, Fechner H, Poller W, Planz O, Kurth R, Norley S. Vaccine protection against lethal homologous and heterologous challenge using recombinant AAV vectors expressing codon-optimized genes from pandemic swine origin influenza virus (SOIV). *Vaccine* 2011; 29:1690-9; PMID:21195079; <http://dx.doi.org/10.1016/j.vaccine.2010.12.037>
- [224] Balazs AB, Bloom JD, Hong CM, Rao DS, Baltimore D. Broad protection against influenza infection by vectored immunoprophylaxis in mice. *Nat Biotechnol* 2013; 31:647-52; PMID:23728362; <http://dx.doi.org/10.1038/nbt.2618>
- [225] Limberis MP, Adam VS, Wong G, Gren J, Kobasa D, Ross TM, Kobinger GP, Tretiakova A, Wilson JM. Intranasal antibody gene transfer in mice and ferrets elicits broad protection against pandemic influenza. *Sci Transl Med* 2013; 5:187ra72; PMID:23720583; <http://dx.doi.org/10.1126/scitranslmed.3006299>
- [226] Marr L, Lülff AT, Freudenstein A, Sutter G, Volz A. Myristoylation increases the CD8+ T cell response to a GFP prototype antigen delivered by modified vaccinia virus Ankara. *J Gen Virol* 2016; 97(4): 934-40; <http://dx.doi.org/10.1099/jgv.0.000425>; PMID:26864442
- [227] Alharbi NK, Spencer AJ, Salman AM, Tully CM, Chinnakannan SK, Lambe T, Yamaguchi Y, Morris SJ, Orubu T, Draper SJ, et al. Enhancing cellular immunogenicity of MVA-vectored vaccines by utilizing the F11L endogenous promoter. *Vaccine* 2016; 34:49-55; PMID:26616553; <http://dx.doi.org/10.1016/j.vaccine.2015.11.028>