Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Xenogenic Adenoviral Vectors

19

Suresh K. Mittal[1], Yadvinder S. Ahi[2], Sai V. Vemula[3]
[1]Department of Comparative Pathobiology, College of Veterinary Medicine and Purdue University Center for Cancer Research, Purdue University, West Lafayette, IN, USA; [2]HIV Drug Resistance Program, National Cancer Institute, Frederick National Laboratory for Cancer Research, Frederick, MD, USA; [3]Laboratory of Molecular Virology, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, MD, USA

1. Introduction

Invention of novel vectors for gene delivery into a host has garnered great interest among the scientific community. The foremost applications for gene delivery include the development of vaccines and therapeutics for several infectious or genetic diseases and cancers. For the success of a gene delivery platform, the two most important criteria are safety and efficiency. A tremendous increase in the knowledge of molecular biology, genomics, proteomics, and immunology from 1980 to 2014 has allowed remarkable progress in recombinant DNA technology, which has greatly influenced the development of novel vector systems both viral and nonviral.

While clinical trials for gene therapy and vaccine applications have seen utilization of both viral and nonviral vectors, a significant majority of clinical trials have relied on viral vectors primarily due to the following reasons: (1) Viral vectors are naturally well adapted to infect the host due to the coevolution of viruses and host, thus enabling efficient delivery of the target gene into host cells; (2) Expression of the antigen in host cells allows for the presentation of antigenic peptides on MHC class I resulting in stimulation of cellular immune response; (3) Some viral vectors can infect antigen-presenting cells such as dendritic cells and macrophages allowing for direct presentation of antigenic peptides on both MHC class I and MHC class II and (4) Viral proteins themselves can act as immune stimulants providing a strong adjuvant effect which can boost the immune response to the antigen.[1–4] The most commonly used viral vectors in clinical trials are based on adenoviruses (AdVs), retroviruses, poxviruses, and herpesviruses. Of all gene therapy clinical trials, AdV vectors are currently the most commonly used gene delivery platform (http://www.abedia.com/wiley/vectors.php).

AdVs were first isolated more than 60 years ago from human adenoid tissues undergoing spontaneous degeneration in tissue culture.[5] Although the first evaluation of AdVs as an antitumor agent dates back to 1956,[6] it was not until the advent of recombinant DNA technology during the early 1980s that the therapeutic potential of AdVs was recognized, and AdV vectors were first developed. Since then, AdV biology has been studied thoroughly, and AdV vectors have been greatly optimized for various applications including vaccine vectors, gene therapy, oncolytic cancer therapy, and cancer immunotherapy.

Adenoviral Vectors for Gene Therapy. http://dx.doi.org/10.1016/B978-0-12-800276-6.00019-X

AdVs are nonenveloped viruses with icosahedral capsids varying in size from 70 to 90 nm with the dsDNA genome ranging from 26 to 46 kb.[7] AdVs are classified under the family *Adenoviridae,* which is further divided into five genera: *Mastadenovirus*, *Aviadenovirus*, *Atadenovirus*, *Ichtadenovirus*, and *Siadenovirus*.[8] The currently known 57 serotypes of human AdVs (HAdVs) encompass species A through G based on genome sequence.[9] AdV capsids are primarily composed of the major capsid proteins, the hexon, the fiber, and the penton base which determines the tropism and immunogenicity of the virus. The relatively less abundant proteins in AdV capsids are called minor capsid proteins and include the core proteins (Tp, V, VII, and mu) and the cement proteins (IIIa, VI, VIII, and IX). Depending on the species type, AdVs use a variety of receptors for cell entry and include the coxsackievirus-adenovirus receptor (CAR), CD46, VCAM-1, CD80, CD86, heparin sulfate proteoglycans, desmoglein-2, and others.[10,11]

2. Advantages of Adenovirus Vectors

AdV-derived vectors fulfill many of the desirable features of a gene delivery platform. Although AdVs are known to infect a wide range of species, they rarely cause a serious disease in an immune-competent host. Most HAdVs are associated with self-limiting disease in humans and may manifest as mild upper respiratory tract infections, conjunctivitis, gastroenteritis, or ear infections, depending on the HAdV species involved. In most instances, the severity of the disease is a function of the host's immune function.[7] Their relatively innocuous nature makes AdVs particularly suitable for developing into a safe vector system. AdV vectors offer several other advantages including (1) the ability to infect a wide range of dividing and nondividing mammalian cells; (2) a robust transgene expression that can be restricted to a specific tissue by employing a tissue specific promoter; (3) the fact that they are easy to generate and can be grown to high titers; (5) their inability to integrate into host genome with a minimal risk of insertional mutagenesis; (6) the induction of strong humoral and cellular adaptive immune response to the transgene; and (7) strong activation of the innate immune response to provide an adjuvant effect that can potentiate booster immunity and may be desirable in vaccine applications.[12]

3. Preexisting Adenovirus Immunity

Among the known HAdV serotypes, the biology of the subgroup C serotype HAdV-5 is the most thoroughly studied; therefore, HAdV-5-derived vectors were the first to be evaluated in preclinical studies. The HAdV-5 vectors show great promise for gene delivery due to extremely strong transgene expression, a highly optimized system for production and preparation on a large scale, and their ability to induce strong humoral and cellular immune responses in nonhuman primates as well as in rodents.[13,14] As a result, the majority of the initial clinical studies involving AdV vectors rely on

HAdV-5 vectors.[15] The high prevalence of preexisting AdV immunity in humans has been noted as a major concern in the success of HAdV-5 vectors in clinical studies.

The development of preexisting AdV immunity is mainly due to exposure of a majority of the human population worldwide to one or more HAdV serotypes from natural infections resulting in strong anti-HAdV humoral and cellular immune responses.[16–19] Given that the natural exposure to HAdVs can be highly variable, the level of preexisting AdV immunity is expected to vary among individuals and human populations making the efficacy of HAdV vectors in clinical trials unpredictable. The high immunogenicity of AdV vectors implies that a first inoculation with an AdV vector will result in AdV serotype-specific immune responses that will preclude subsequent use of the same AdV serotype. Thus, high efficacy of any single AdV serotype is not expected even in individuals lacking preexisting AdV immunity.

The humoral immune response to HAdVs consists of both neutralizing and non-neutralizing antibodies.[20] Although the anti-HAdV-neutralizing antibodies (nAbs) targeting the three major capsid proteins, hexon, penton, and fiber, have been confirmed,[21,22] the anti-hexon nAbs are the most significant in vector neutralization. The major epitopes for anti-hexon nAbs are located in the exposed loops, also known as hypervariable regions, located on the surface of the virus particle.[23] The nAbs bind to the AdV vector in the blood, preventing its binding to the cell surface and subsequent internalization, thus resulting in rapid clearance from the host. The end result is a drastic reduction in transgene expression and an induction of transgene-specific immune responses. Such blunting of the efficacy of HAdV-5 vectors in the presence of preexisting immunity has been demonstrated by generating artificial serotype-specific immunity in animals preinoculated with HAdV-5.[24–26] The nonneutralizing, but cross-reactive, anti-HAdV antibodies are generated after repeated administration or infections with the same AdV serotype.[26] The nonneutralizing antibodies can also significantly reduce the transgene expression through Fc receptor-dependent and -independent mechanisms resulting in poor efficacy of the AdV vector.[27]

The cellular component of preexisting AdV immunity consists of both $CD4^+$ and $CD8^+$ T cells that are highly cross-reactive and widely prevalent in human populations.[28] The epitopes recognized by anti-AdV T cells are located primarily in the hexon and are highly conserved among various HAdV serotypes,[29–33] implying that exposure to a single HAdV serotype may hamper the efficacy of other HAdV serotypes. Studies have revealed that HAdV-5-specific T cells are prevalent in 80–100% of human subjects from Europe and the United States.[32,34,35] More importantly, HAdV-specific T cells may be prevalent even in the absence of nAbs.[20,35] The presence of vector-specific CD8 T cells causes cytolysis and elimination of the vector-transduced cells, while the $CD4^+$ T cells will potentiate the humoral immune response to the vector.

In addition to the development of adaptive immune responses, AdV vectors can also induce an immune activation characterized by overproduction of proinflammatory cytokines, chemokines,[36–38] high levels of type I interferons (IFNs),[39] complement activation, and phagocytosis.[40–42] The pattern recognition receptors (PRR) involved in the recognition of AdV vectors receptors include Toll-like

receptors (TLR) 4 and 9, nucleotide-binding oligomerization domain-like receptors, Factor X, and retinoic acid-inducible gene 1.[28,43] Binding of AdVs to PRR results in the activation of downstream signaling pathways such as NF-κB culminating in the production of proinflammatory mediators including tumor necrosis factor alpha (TNFα), IL-1, IL-6, IL-8, IL-10, IL-12, IFN-γ, granulocyte colony-stimulating factor, macrophage inflammatory protein (MIP)-1α/β, IP-10, RANTES, and monocyte chemoattractant protein-1.[28,41] The AdV-induced innate immune response may be beneficial for targeted cancer gene therapy and vaccine applications where the goal is to eliminate the AdV vector-transduced cells and generate a robust immune response. However, activation of innate immune responses can result in rapid destruction of AdV-transduced cells, thus reducing the transgene expression. Furthermore, intravenous inoculations of large doses of AdV vectors can also have life-threatening consequences mediated by strong activation of innate immune reactions.[44]

The tremendous potential of AdV vectors is hampered by the immune responses to AdVs. For the success of these vectors, it is critical to understand thoroughly the underlying mechanisms. Several strategies have been developed to circumvent the preexisting immunity and minimize the innate immune reactions to AdVs. They can be broadly classified into either genetic or chemical modifications. The genetic modifications include hexon pseudotyping, hexon HVR swapping, fiber pseudotyping, fiber truncation, use of alternative trimerization motifs for fiber, and the use of vectors derived from rare HAdV serotypes or nonhuman AdVs.[12,45–48] The chemical modifications include derivation of AdV vectors with polyethylene glycol or cationic polymers such as poly-L-lysine, *N*-[2-hydroxypropyl] methacrylamide, arginine-grafted bioreducible polymers, coating of AdV vectors with cationic liposomes, and lipidic envelopes.[12,46,47] This review will focus on AdV vectors derived from nonhuman AdVs.

4. Nonhuman Adenovirus Vectors

The idea of developing vectors derived from nonhuman AdVs is based on the premise that nonhuman AdVs are less prevalent and consequently will have lower seroprevalence among human populations. Similar to the HAdVs, the nonhuman AdVs do not cause serious disease in their natural host. Preexisting immunity to nonhuman AdVs can be expected only in their natural hosts. In addition, the overall genome organization and structural features of nonhuman AdVs are not vastly different from the HAdVs; vector design strategies and techniques used in working with HAdV vectors could, in principle, be applied to the nonhuman AdV vectors. The initial impetus for developing nonhuman AdV vectors was seen in the early 1990s when the limitations of HAdV vectors were starting to become evident. Since then gene expression vectors based on various species of nonhuman AdVs have been constructed and extensively characterized. These include simian AdV serotypes (SAdVs), bovine AdV serotype 3 (BAdV-3), porcine AdV serotypes 3 and 5 (PAdV-3 and PAdV-5), ovine AdV serotype 7 (OAdV-7), canine AdV serotype 2 (CAdV-2), murine AdV serotype 1 (MAdV-1),

and fowl AdV serotypes (FAdVs). In this review, various aspects of these nonhuman AdV vectors including strategies for construction, types of vector/s, genome organization (Figure 1), insertion sites, foreign gene insertion capacity, receptors, tropism, impact of preexisting immunity, and preclinical and clinical studies will be discussed. Examples of nonhuman AdV-based vectors as gene delivery vehicles for recombinant vaccines or gene therapy applications are shown (Table 1).

4.1 Simian Adenovirus Vectors

The first SAdV vector developed was a chimpanzee AdV (chAdV)-based vector derived from chAdV-68 (SAdV-25) by homologous recombination of a shuttle vector and vector genome in the 293 cell line expressing HAdV-5 early region 1 (E1) proteins. It appears that the E1 of chAdV-68 is closely related to the E1 of HAdV-5 and thus allows for efficient replication of the E1-deleted chAdV-68.[49] This similarity offers two advantages: (1) there is no need to create cell lines expressing E1 from a chAdV and (2) there is less chance of vector contamination with a replication-competent AdV due to variation in the common sequences of the chAdV-68 vector and HAdV-5 E1. In addition, there are substantial variations in the hypervariable region within the hexon sequence of chAdV-68 and several HAdVs. Given that the hypervariable regions of the hexon carry the type-specific epitopes for HAdV-neutralizing antibodies,[50] it is not surprising that chAdV-68 is not cross-neutralized by HAdV preexisting immunity.

The AB loop within the knob domain of chAdV-68 fiber, which mediates binding of CAR-binding HAdV serotypes, is identical to HAdV-4 and is very similar to HAdV-2, HAdV-5, and HAdV-12, thus explaining the observation that CAR serves as a receptor for chAdV-68.[51] Other chAdVs such as chAdV-6 (SAdV-23) and chAdV-7 (SAdV-22) are closely related to subgroup E HAdVs; chAdV-68[52] may possibly use CAR as a receptor for cell entry. The chAdV-1 (SAdV-21), which is more closely related to subgroup B HAdVs, utilizes CD46 as a receptor for cell entry.[53] It is conceivable that the tropism of chAdV vectors utilizing CAR or CD46 as receptors would be similar to the CAR or CD46 binding HAdV serotypes.

Depending on the E1 deletion with or without early region 3 (E3) deletion, the chAdV-68 vectors allow for transgene lengths of up to 5000 or 1600 bp respectively, without significant decreases in virus yields or virus particle-to-PFU ratios compared to smaller size transgenes.[54] The E1- and E1/E3-deleted chAdV-68 vectors are comparable in terms of the level of transgene expression and induction of transgene-specific $CD8^+$ T cell response. However, incorporation of an E4 deletion results in decreased transgene expression and $CD8^+$ T cell response. Interestingly, the level of protein expression by the chAdV-68 vector seems to be a function of transgene length. A chAdV-68 vector expressing simian immunodeficiency virus (SIV) gag alone resulted in much higher protein expression than a vector expressing SIV gag–pol or SIV gag–pol–nef fusion proteins, and, consequently, resulted in a higher gag-specific $CD8^+$ T cell response.[54] It is likely that the competition of peptides for the MHC class I peptide-binding site decreased the overall magnitude of immune response to gag. Thus, even though the chAdV-68 vectors are capable of incorporating large inserts, the choice of transgene length should be carefully evaluated.

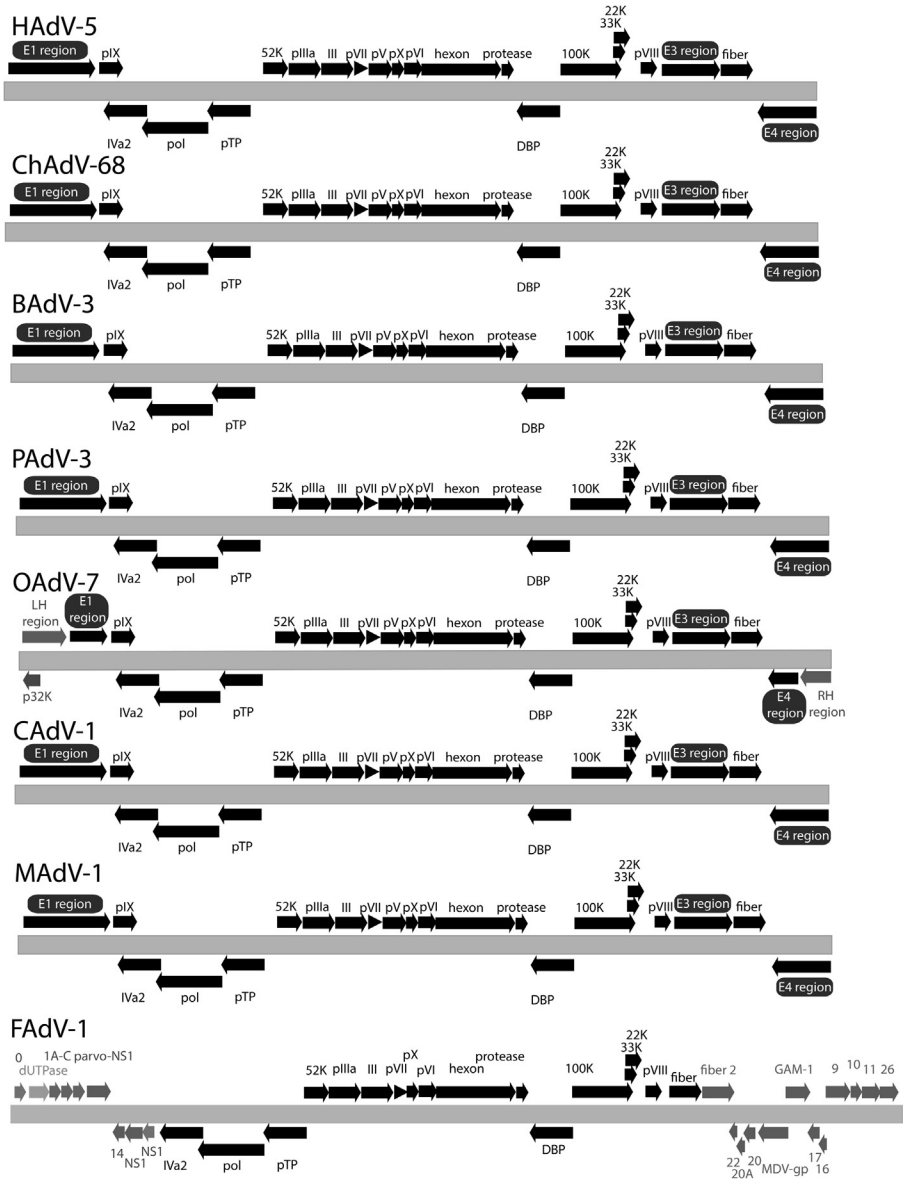

Figure 1 Simplified depiction of gene arrangements of simian adenovirus (AdV) (chAdV-68),[a] bovine AdV (BAdV-3),[105] porcine AdV (PAdV-3),[201] canine AdV (CAdV-1),[154] ovine AdV (OAdV-7),[b] murine AdV (MAdV-1),[c] and avian AdV (FAdV-1)[d,196] compared to human AdV (HAdV-5).[e] The conserved genes are indicated with black arrows. The early regions (E) 1 (E1), E3, and E4 are depicted with black boxes, respectively; however, the variable features of E1, E3, and E4 regions in various AdVs are not shown. The unique genes are shown with dark or light gray arrows. The maps are not at scale. [a]Roy S, Gao G, Clawson DS, Vandenberghe LH, Farina SF, Wilson JM. Complete nucleotide sequences and genome organization of four chimpanzee adenoviruses. *Virology* 2004; 324:361–72.

Preexisting immunity. The chAdVs show great potential as gene therapy and vaccine vectors and are the only nonhuman AdV vectors that have progressed to clinical trials to date. The chAdVs have little to no seroprevalence in the human population worldwide which makes them suitable vector candidates for use in humans.[55,56] In the hexons of chAdV and HAdVs,[57] there is very little sequence similarity in the hypervariable region, which is the location of the major neutralization determinant, suggesting that the chAdV can circumvent preexisting immunity against HAdV vectors. A low frequency of nAbs against chAdV, ranging from 0 to 4% has been observed in humans in the United States, Europe, Thailand,[58] and China,[59,60] whereas nAbs to HAdV serotypes may reach up to 30–45% in these countries. Up to ~20% of sera from sub-Saharan countries have been found to be positive for nAb against chAdV, whereas ~60–80% sera are positive for nAb against HAdV serotypes.[58,61,62] In Brazil, the seroprevalence rates of chAdV nAbs are between 20 and 23%; HAdV nAbs were between 40 and 70% depending on the serotype.[16,56] Therefore, even though the seroreactivity to chAdVs is higher in sub-Saharan countries and Brazil than the rest of the world, it is still lower than that of HAdVs.

Neutralizing antibody titers of >200 are considered to hamper the efficacy of AdV vectors[63]; therefore, it is important to consider the titers of chAdV nAbs. As anticipated, 10% or less of human sera with chAdV nAb had titers of >200, whereas about 40% of the human sera positive for HAdV nAbs had titers of >200. The lower titers of chAdV nAbs, in combination with the lower overall seroreactivity compared to HAdV serotypes, suggest that chAdV serotypes are less likely to suffer from preexisting immunity than HAdV serotypes.

Immunological potential. The chAdV vectors belonging to group C of HAdV species have the highest immunogenic potential among all chAdV vectors.[57] In sub-Saharan Africa where the target population for endemic diseases such as human immunodeficiency virus (HIV), malaria, and tuberculosis exists, it is particularly important for a vaccine to have the ability to induce a $CD8^+$ T cell response.[64] The most effective HAdV serotypes (HAdV-5 and HAdV-26) in generating a $CD8^+$ T cell response would be inefficient in these areas owing to modest to high levels of preexisting immunity. The other HAdV serotype, HAdV-35, which has the least preexisting immunity worldwide, is not as effective as HAdV-5 and HAdV-26 in generating a $CD8^+$ T cell response in particular and overall immunogenicity in general.[19,65,66]

◀ [b]Kumin D, Hofmann C, Rudolph M, Both GW, Loser P. Biology of ovine adenovirus infection of nonpermissive cells. *J Virol* 2002; 76:10,882–93. [c]Hemmi S, Vidovszky MZ, Ruminska J, Ramelli S, Decurtins W, Greber UF, et al. Genomic and phylogenetic analyses of murine adenovirus 2. *Virus Res* 2011; 160:128–35. [d]Marek A, Kosiol C, Harrach B, Kaján GL, Schlötterer C, Hess M. *Vet Microbiol* 2013; 166:250–56; and Harrach B, Benko M, Both GW, Brown M, Davison AJ, Echavarria M, et al. The Double Stranded DNA Viruses: Adenoviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. *Virus taxonomy: ninth report of the international committee on taxonomy of viruses*. Waltham (MA): Academic Press: Elsevier; 2011. p. 125–41. [e]Chroboczek J, Bieber F, Jacrot B. The sequence of the genome of adenovirus type 5 and its comparison with the genome of adenovirus type 2. *Virology* 1992; 186:280–85.

Table 1 Examples of Nonhuman Adenovirus (AdV)-Based Vectors as Gene Delivery Vehicles for Recombinant Vaccines or Gene Therapy Applications

Adenovirus (AdV)	Vector Type	Insertion Site	Pathogen/Cancer/Gene Therapy	Antigen	References
Simian AdVs					
chAdV-3, chAdV-63	E1, E3 deleted	E1	Simian immunodeficiency virus	Gag	67
chAdV-6, chAdV-68	E1 deleted	E1	Human immunodeficiency virus	Gag	68
chAdV-7	E1, E3 deleted	E1	Ebola	Env GP	72
chAdV-7	E1, E3 deleted	E1	Respiratory syncytial virus	Fusion protein	75
chAdV-68	E1 deleted	E1	Rabies	Glycoprotein	76,77
chAdV-7	E1 deleted	E1	H5N1 influenza	NP	78
PanAdV-3	E1, E3 deleted	E1	H5N1 influenza	NP-M1 fusion	79
chAdV-7	E1 deleted	E1	Severe acute respiratory syndrome (SARS)	Spike protein	83
chAdV-63	E1, E3 deleted	E1	Human immunodeficiency virus	HIV*cons*	89[a]
chAdV-63	E1, E3 deleted	E1	*P. falciparum*	ME-TRAP	96
chAdV-63	E1, E3 deleted	E1	*P. falciparum*	AMA1	97
chAdV-63	E1, E3 deleted	E1	*P. falciparum*	MSP1	98
chAdV-3	E1, E3 deleted	E1	Hepatitis C virus	NS	104
chAdV-3	E1, E3 deleted	E1	Colon carcinoma	CEA	69
Bovine AdVs					
BAdV-3	E3 deleted	E3	Bovine herpes virus-1	Glycoprotein D (gDt)	126
BAdV-3	E3 deleted	E3	Bovine herpes virus-1	gDt	127
BAdV-3	E3 deleted	E3	Bovine viral diarrhea virus	Glycoprotein E2 (gE2)	128
BAdV-3	E3 deleted	E3	Bovine respiratory syncytial virus	Glycoprotein G (gG) and IL-6	129
BAdV-3	E1, E3 deleted	E1	A/Hong Kong/156/97 (H5N1)	Hemagglutinin (HA)	131
BAdV-3	E3 deleted	E3	Bovine herpes virus −1 and bovine respiratory syncytial virus	gG and gDt	130

Porcine AdVs					
PAdV-3	E3 deleted	E3	Classical swine fever virus	gp55/E2 gene	136
PAdV-3	E3 deleted	E3	Classical swine fever virus	gp55/E2 gene (DNA/PAdV-3)	137
PAdV-3	E3 deleted	E3	Pseudorabies virus (PRV)	Glycoprotein D (gD)	138
PAdV-3	E1, E3 deleted	E1	A/Hanoi/30408/2005 (H5N1)	HA	139
PAdV-5	E3 deleted	E3	Transmissible gastroenteritis virus	Spike protein	141
Ovine AdVs					
OAdV-7	NA	Site III	Hepatitis C virus	Nonstructural protein 3 (NS3)	146
OAdV-7	NA	Site II	*Taenia ovis*	45W	147
OAdV-7	NA	Site III	Human immunodeficiency virus 1	Gag	148
OAdV-7	NA	Site II	Skeletal muscle gene therapy	Human alpha-1 anti-trypsin (hAAT)	150
OAdV-7	NA	Site III	Prostate cancer	*E. coli* purine fludarabine phosphorylase (PNP)	152
Canine AdVs					
CAdV-1	E3-deleted	E3	Canine parvovirus	Capsid	156
CAdV-2	cRAd		Canine osteosarcoma		161
CAdV-2	Gutless		Canine mucopolysaccharidosis VII	Human GUSB	172–174
Murine AdVs					
MAdV-1	cRAd		Murine colon carcinoma	mGM-CSF	189
Avian AdVs					
FAdV-10	NA	Near right ITR	Infectious bronchitis virus	VP2	197
FAdV-8	NA	Near right ITR	Infectious bronchitis virus	Spike peplomer S1 subunit (S1)	199

[a]Ondondo B, Brennan C, Nicosia A, Crome SJ, Hanke T. Absence of systemic toxicity changes following intramuscular administration of novel pSG2.HIV*consv* DNA, ChAdV63.HIV*consv* and MVA.HIV*consv* vaccines to BALB/c mice. *Vaccine* 2013; 31:5594–601.

In HIV, both chAdV-derived vectors have markedly lower seroreactivity titers than HAdV-5, HAdV-26, and HAV-35 across populations in southern Africa, eastern Africa, central Africa, India, the United States, South America, and the Caribbean.[67] In addition, chAdV vectors result in the gag-specific $CD8^+$ T cell response and expression levels of IFN-γ, IL-2, and TNFα that are comparable to or better than those of the HAdV serotypes at a wide range of doses.[67] The chAdV vectors are also found to induce the SIV gag-specific $CD4^+$ response better than HAdV-5.[67]

In addition, chAdV-3 is found to be as effective as HAdV-5 in boosting a DNA or a HAdV-28 primed SIV gag-specific $CD8^+$ T cell response.[67] A heterologous prime–boost regimen with chAdV vectors carrying the HIV-1 gag gene results in strong and robust $CD8^+$ T cells in various systemic compartments including the genital tract with intramuscular immunization within 2 weeks.[68] Moreover, the $CD8^+$ T cell response seems to be long lasting with the gag-specific $CD8^+$ T cell being detected in all systemic compartments for up to one year after immunization.[68] These observations suggest that chAdV-based vectors can be used for developing vaccines against infections requiring the $CD8^+$ T cell-mediated immune (CMI) response.

A single immunization with a chAdV vector expressing HIV-1 gag elicited a potent antigen-specific cellular immune response [~2000 IFN-γ spot-forming cells (SFCs) per million PBMCs] that contracted eventually but persisted beyond 5 years (~400 IFN-γ SFCs per million PBMCs). A booster at this 5 year stage with a chAdV vector carrying the same antigen resulted in a rapid increase in the number of the gag-specific IFN-γ producing T cells. Additionally, gag-specific antibodies could be detected before the booster and increased by a factor of 10 after the booster,[57] implying that chAdV vectors are capable of inducing long-lasting T and B cell memory.

A recent report compared the immunological potency of chAdV-3 with that of HAdV-5 as an anticancer vaccine vector.[69] The chAdV-3-based vector, expressing transgene at levels similar to that of HAdV-5, was able to induce higher levels of a $CD8^+$ IFN-γ-positive T cell response at significantly lower doses compared to a HAdV-5-based vector. Additionally, a stronger immune response was observed in a chAdV-3/chAdV-3 prime–boost regimen than either a HAdV-5/HAdV-5 or a HAdV-5/chAdV-3 prime–boost regimen.[69] Most importantly, the chAdV-3-derived vector was able to confer antitumor protection in mice with anti-HAdV-5 immunity.[69]

Another study[70] reported that a chAdV-23 (also known as SAdV-22 or Pan-5)-derived vector was equally or more efficient than an HAdV-5-derived vector in transducing low-passage brain tumor cells, $CD133^+$ and $CD133^-$ glioma tumor stem cells derived from human patients. Given that the $CD133^+$ cells are difficult to treat with traditional chemotherapy, the aforementioned observations warrant testing of SAdV-23-based vectors as a treatment for human brain tumors.

The chAdV-based vectors are not only capable of inducing robust humoral and cellular adaptive immune responses but also have been shown to induce protective immunity against a variety of infections including Ebola virus in mice and nonhuman primates,[71–74] respiratory syncytial virus in neonates,[75] rabies virus,[76,77] H5N1 influenza virus,[78,79] malaria,[64,80,81] Rift Valley fever virus,[82] and severe acute respiratory syndrome virus.[83] The ability to induce protective immunity in human populations in the presence of preexisting immunity to HAdV serotypes is a highly desirable trait

of chAdV-based vectors. These vectors have the potential to develop into a common platform for vaccine or gene therapy purposes.

4.1.1 Chimpanzee Adenovirus Vectors in Clinical Trials

HIV-1. In a heterologous prime–boost regimen, a chAdV-63 vector in combination with a plasmid DNA or modified vaccinia vector Ankara (MVA) as a vaccine for delivering HIV*consv* was evaluated in uninfected human subjects.[84] The HIV*consv* is based on the 14 functionally most conserved subprotein domains of HIV-1 and is common to most virus variants worldwide.[85–88] The combinatorial regimen that included the chAd63.HIV-*consv* resulted in an HIV*consv*-specific IFN-γ-producing T cells with remarkably high frequencies. Furthermore, both $CD4^+$ and $CD8^+$ T cells with broad specificity and multiple intercellular signaling molecules were induced. In addition, the effector T cells which were found to be specific for gag and pol were efficacious in inhibiting HIV-1 replication in cultured autologous $CD4^+$ T cells by a factor of 5.79 log. No adverse side effects were reported in any of the subjects receiving the vaccine formulation.[84] A follow-up Phase I clinical trial HIV-CORE 002 with the same combinatorial regimens discussed above did not report significant safety or tolerability concerns.[89] Only mild-to-moderate local reactogenicity and systemic effects such as mild pain, erythema, fever, and headache lasting for fewer than 3 days postadministration were described in the majority of vaccines receiving chAd63.HIV*consv*. Following these encouraging results, a Phase I clinical trial using chAd63.HIV*consv* and MVA.HIV*consv* is underway (ClinicalTrials.gov; NCT01712425).

Malaria. Malaria is caused by *Plasmodium falciparum* and is a major pathogen responsible for childhood morbidity and mortality in Africa and other countries. The primary objective of an effective malaria vaccine will be to control the disease transmission. Multiple preclinical studies have demonstrated a strong correlation between $CD8^+$ T cells and protective efficacy.[90–94] However, the existing subunit vaccines are not good enough to induce high levels of malaria antigen-specific $CD8^+$ T cells.[95]

Several studies have shown that heterologous prime–boost immunization with chAdV-63 and MVA expressing ME-TRAP, a liver stage antigen of *P. falciparum*, are capable of inducing robust $CD8^+$ T cell response and protection.[64,96–98] The chAd63.ME-TRAP and MVA.ME-TRAP did not cause any significant local or systemic adverse effects.[96] The chAd63.ME-TRAP demonstrated a good safety profile in individuals in Kenya, Gambia, and United Kingdom [96,99] with a dose of up to 2×10^{11}.[99] The chAd63.ME-TRAP and MVA.ME-TRAP prime–boost regimen induced high frequencies of antigen-specific IFN-γ secreting T cells, reaching beyond 2000 SFCs/million PBMCs in two independent Phase Ib clinical trials.[96] The induced cellular immune response included cytokine secreting, antigen-specific $CD4^+$ and $CD8^+$ T cell populations.[64] In addition, the response seemed to be long lasting since it had not significantly decreased at 9 months postvaccination.[96] The high levels of antigen-specific IFN-γ secreting T cells are highly remarkable as it is difficult to induce more than 1000 SFCs/million PBMCs.[64,96] ChAd63.ME-TRAP priming followed by MVA.ME-TRAP boosting induced high levels of IgG response to the TRAP antigen. Importantly, the low levels of anti-chAd63 nAb titers detected pre- and postadministration of chAd63.ME-TRAP did not attenuate the immunogenicity of the vaccines. Finally, the chAd63.ME-TRAP and

MVA.ME-TRAP prime–boost also provided sterile to partial protection against controlled human malaria infection with a heterologous malaria strain, thereby demonstrating strong correlation between immunogenicity and protective efficacy.

Another heterologous prime–boost vaccine regimen employed the chAd63 and MVA encoding for either one or both of the blood stage malaria antigens AMAI and MSPI, alone or in combination with the liver stage antigen ME-TRAP.[97] The chAdV-63 vectors displayed an excellent safety profile and induced significantly higher T cell responses compared to other delivery platforms tested (DNA/MVA or Fowl Pox virus/MVA). The cellular immune response was broadly specific to the AMA1 and MSP1, persisted at high levels, and comprised of relevant cytokine secreting $CD8^+$ and $CD4^+$ T cell populations. The chAdV-63 vectors also induced an antigen-specific IgG response.[97,98] The chAdV-63-MVA prime–boost combination is now a commonly employed and versatile vaccine delivery system capable of inducing strong cellular and humoral immune responses.

Hepatitis C. Hepatitis C virus (HCV) is another pathogen of immense public health significance against which the chAdV-derived vectors have been used for vaccine delivery. HCV is capable of establishing persistent, stealthy infection in immunocompetent hosts in which cirrhosis has affected a significant portion of the liver. The current treatments are becoming increasingly effective but are costly and have serious side effects.[100]

Induction of high levels of virus-specific $CD4^+$ and $CD8^+$ T cells is important for protection against HCV,[101–103] implying that a successful HCV vaccine will be able to mount a strong and long-lasting T cell response. A chAdV-3-based vector, alongside the rare HAdV serotype of HAdV-6, expressing the NS proteins of HCV genotype 1B was recently tested in a Phase I clinical trial.[104] Both vectors were found to be overall safe and well tolerated, resulting in only mild local and systemic side effects at various doses ranging from 5×10^8 to 7.5×10^{10} vp. The chAdV-3 vector used at 2.5×10^{10} vp dose induced more than 1000 IFN-γ SFCs/million PBMCs—a level similar to or higher than the HAdV-6 vector. Furthermore, both viral vectors were able to induce broad T cell responses, targeting peptides encompassing multiple viral gene products including NS3, NS4A/B, NS5A, and NS5B. The induced T cell responses for each vector included both $CD4^+$ and $CD8^+$ T cells with the $CD8^+$ response being the more dominant of the two. T cell populations secreting IFN-γ/TNFα, IFN-γ/IL-2, or all three cytokines were detected with a minimal IL17A response. In addition, the induced T cells responses were found to be cross-reactive with other HCV genotypes 1A, 3A, and 1B with genotype 1B representing the immunogen. Finally, detectable and functional memory T cell could be observed up to one year postboost, suggesting that the vaccines induced a long-lasting T cell response.[104]

In the same clinical trial, chAdV-3 and HAdV-6 were also tested in a heterologous prime–boost regimen. The chAdV-3 prime/HAdV-6 boost regimen, in contrast to the HAdV-6 prime/chAdV-3 boost regimen, resulted in a stronger boost response that was also consistent among different vaccines. However, the boost response was still not as high as that observed for the boost in chAdV-63/MVA malaria trials. The poorer chAdV-3 prime/HAdV-6 boost response is surprising given the strong and functional responses at priming. The authors proposed that the nAbs induced on priming were possibly interfering with the heterologous vector used at priming and resulted in attenuation of AdV vector-specific and NS antigen-specific T cell responses. Nonetheless,

the overall findings of this clinical trial of a T cell-based HCV vaccine have again demonstrated the utility of chAdV vectors as a vaccine delivery platform.

4.2 Bovine Adenovirus-Based Vectors

AdVs infecting cattle, termed as bovine AdVs (BAdVs), have been classified under the two genera, *Mastadenovirus* and *Atadenovirus*. Ten serotypes of BAdVs (BAdV-1–10) have been isolated thus far, and most of these are responsible for mild diseases of the gastrointestinal or respiratory tracts in bovines. Of the 10 BAdV serotypes, BAdV-3 is the best characterized. The complete nucleotide sequence and genome map of BAdV-3 is available.[105] The BAdV-3 genome shares a high level of similarity with HAdV-5 with certain differences. The E3 region in BAdV-3 is relatively smaller and less complex compared to the corresponding E3 region from HAdV-5.[106] BAdV-3 E1 proteins (E1A, E1B-157R, and E1B-420R) show functional or amino acid sequence homologies with their counterpart E1 proteins of HAdV-5 and BAdV-3. E1A complements HAdV-5 E1A functions[107,108] suggesting functional similarities between BAdV-3 E1 and HAdV-5 E1. Structurally, the BAdV-3 fiber is exceptionally long and bent at several sites[109] indicating that the bending may be necessary to allow the penton base to make contact with the secondary receptors on the cell surface.

Cotton rats (*Sigmodon hispidus*) can serve as a replication-competent small-animal model for evaluating the pathogenesis and vaccine efficacy of BAdV-3 vectors.[110,111] In the presence of circulating BAdV-3-neutralizing antibodies, intranasal inoculation of cattle with BAdV-3 results in inapparent infection[112] suggesting that a BAdV-3-based vaccine would be successful.

BAdV-3-based vectors have been created using homologous recombination in both mammalian cells[113] and bacteria[114] in addition to I-*Sce*I-based approaches.[105,115,116] Several bovine cell lines have been used to rescue BAdV-based vectors. These include MDBK, BHH3 (bovine human hybrid cell lines expressing HAdV-5 E1),[117] FBRT-HE1 (fetal bovine retinal cells expressing HAdV-5 E1[118] and VIDO R2 (fetal bovine retinal cells expressing HAdV-5 E1).[115] Initially BAdV-3 as a replication-competent vector containing the firefly luciferase gene in the E3 region was constructed in 1995,[113] and the BAdV-3 vector-infected 293 cells efficiently expressed luciferase, suggesting the suitability of BAdV-3 vectors for gene transfer into human cells.

There are a number of findings that further explore the potential of BAdV-3 as a gene delivery platform. The anti-HAdV-5 or anti-BAdV-3 antibodies raised in rabbits or mice are not cross-virus neutralizing, and the reporter gene expression with HAdV-5-LacZ in BAdV-3-primed mice has been significantly higher ($P > 0.05$) than that obtained in HAdV-5-primed animals.[26] BAdV-3 internalization is independent of the HAdV-5 receptors (CAR and αvβ3- or αvβ5-integrin)[119] but utilizes α(2,3)-linked as well as α(2,6)-linked sialic acid as a major receptor for internalization.[120] Preexisting HAdV-neutralizing antibodies in humans do not cross-neutralize BAdV-3.[108] HAdV-specific CMI response does not cross react with BAdV-3.[121] BAdV-3 has tropism distinct from that of HAdV-5 and efficiently transduces diverse human and nonhuman cells in culture.[108,122] Unlike HAdV-5, BAdV-3 is a strong inducer of TLR4. There is an absence of Kupffer cell depletion with BAdV-3 in mice[123] while Kupffer

cell depletion with HAdV-5 is the main reason for a faster vector depletion from the host. Intravenous inoculation with a BAdV-3 vector efficiently transduces the heart, kidney, lung, liver, and spleen. The vector persists for a longer duration compared to a HAdV-5 vector especially in the heart, kidney, and lung in a mouse model.[122] Sequential administration of HAdV-5 and BAdV-3 vectors overcomes vector immunity in an immunocompetent mouse model of breast cancer.[124] Persistence of the BAdV-3 genome in human and nonhuman cell lines is similar to that of the HAdV-5 vectors.[125] These various findings underscore why BAdV-3 vectors offer an attractive alternative to HAdV vectors for effectively immunizing individuals with high levels of preexisting HAdV immunity with safety aspects similar to those of HAdV-5 vectors.

BAdV-3-based vectors have been successfully used in gene delivery for vaccination purposes in experimental animals. Intranasal vaccination of cotton rats with a replication-competent BAdV-3 vector, BAV3.E3gD, expressing bovine herpesvirus-1 (BHV-1) gDt glycoprotein induced strong gD-specific IgA and IgG immune responses.[126] In a subsequent study, vaccination of calves with BAV3.E3gD induced gD-specific antibody responses in serum and nasal secretions that conferred protection against BHV-1 challenge.[127] A BAdV-3 vector expressing bovine viral diarrhea virus glycoprotein E2 induced E2-specific IgA and IgG in nasal secretions and serum, respectively, in cotton rats following intranasal immunization.[128] A BAdV-3 vector (BAV327) coexpressing bovine respiratory syncytial virus glycoprotein G and bovine IL-6 from the E3 region was developed.[129] A BAdV-3 vector (BAV851) coexpressing bovine respiratory syncytial virus G and BHV-1gDt proteins was generated, and vaccination of cotton rats with this BAV851 induced strong antigen-specific immune responses.[130]

In order to evaluate whether a BAdV vector can effectively elude high levels of preexisting HAdV-5 vector immunity, naïve and HAdV-5-primed mice were immunized with BAd-H5HA (a BAdV vector expressing HA of a H5N1 influenza virus).[131] Even in the presence of very high levels of HAdV-5-specific neutralizing antibody titer (2133 ± 660), no reductions in HA-specific humoral and CMI responses were observed in mice immunized with BAd-H5HA. In the presence of exceptionally high levels of preexisting vector immunity, mice immunized with BAd-H5HA resulted in approximately 2.8-fold higher hemagglutination inhibition (HI) titers or a 2.3-fold higher percentage of HA-specific $CD8^+$ T cells compared to the levels in naïve mice inoculated with HAd-H5HA. The immunization of naïve or HAdV-primed mice with BAd-H5HA resulted in full protection from morbidity and mortality following a potentially lethal challenge with A/Hong Kong/483/97 (H5N1). Furthermore, a heterologous prime–boost regimen comprised of HAd-H5HA priming and boosting with BAd-H5HA elicited a significantly higher HI response compared with HAd-H5HA or BAd-H5HA alone. These results strongly suggest the importance of using BAdV-based vectors as an alternate to HAdV-based vectors for eluding preexisting vector immunity and in a heterologous prime–boost strategy for enhanced immune responses.

4.3 Porcine Adenovirus-Based Vectors

AdVs have been isolated from pigs and termed as porcine AdVs (PAdVs). There are five serotypes of PAdVs currently known to infect swine (PAdV-1–5). Similar to the other

AdVs, PAdVs are prevalent primarily in swine species and are responsible for gastrointestinal disease and multifactorial porcine respiratory disease complexes. Among the five PAdVs, PAdV-3 is the most prevalent and well-characterized serotype. Initially isolated from a healthy young piglet, PAdV-3 is associated with subclinical infection. The complete nucleotide sequence and transcription map of PAdV-3 is available.[105] PAdV-3 shares genomic and structural similarities with HAdV-5. Like HAdV-5, PAdV-3 belongs to the genus *Mastadenovirus*, and its genome consists of five early transcription units (E1A, E1B, E2, E3, and E4).[105] Further, the PAdV-3 E1 transaction unit has been shown to complement the functions of the HAdV-5 E1 transcription unit.

However, unlike HAdV-5, PAdV-3 internalization into cells is independent of CAR and $\alpha_v\beta_3$- or $\alpha_v\beta_5$-integrin receptors; the primary receptors for HAdV-5 and PAdV-3 are distinct.[132] Anti-HAdV-5 or anti-PAdV-3 antibodies raised in rabbits or in mice do not cross-neutralize, and the reporter gene expression with HAdV-5-LacZ in PAdV-3-primed mice show significantly higher P values ($P > 0.05$) than those obtained in HAdV-5-primed animals.[26] Preexisting HAdV-specific neutralizing antibodies in humans do not cross-neutralize PAdV-3.[133] A PAdV-3 vector efficiently transduces a number of human, murine, porcine, and bovine cells in culture,[108,133] suggesting that PAdV-3 vectors are a promising supplement to HAdV vectors.

In studies, the biodistribution of a PAdV-3 vector was comparable to that of a HAdV-5 vector in the mouse model but showed more rapid vector clearance. Only linear episomal forms of PAdV-3 vector genomes were detected in inoculated mice.[10] In addition, PAdV-3-specific T cell responses did not show significant cross-reactivity with HAdV-5 or BAdV-3.[121] Compared to the HAdV-5 vector, the PAdV-3 vector induced higher levels of innate immune responses, including TLRs and proinflammatory chemokines and cytokines.[123] The persistence of a PAdV-3 vector in a number of cell lines was comparable to that of the HAdV-5 or BAdV-3 vectors, and only the linear episomal form of the vector genome was observed.[125] These findings suggest the uniqueness of receptor usage by PAdV-3 and highlights its potential to elude HAdV-specific humoral and CMI responses with a safety similar to that of HAdV-5 vectors.

Construction of recombinant PAdV-3-based vectors has been pursued using homologous recombination in *Escherichia coli* to generate a full-length infectious clone followed by transfection of fetal porcine retinal cell lines transformed with HAdV-5 E1 (FPRT-HE1-5 and VIDO R1).[133,134] Both replication-competent (containing deletion in E3 region) and replication-defective (containing deletions in E1 and/or E3 regions) PAdV vectors have been developed and evaluated as delivery tools for vaccine or gene therapy purposes.[135] A PAdV-3-based classical swine fever virus (CSFV) vaccine (rPAV-gp55) containing the gp55 (E2) gene from the CSFV 'Weybridge' strain into the right-hand end of the PAdV-3 genome has been developed.[136] Transgene expression was driven from the major late promoter and tripartite leader sequences of PAdV-3. Subcutaneous vaccination of outbred pigs with a single dose of the rPAV-gp55 vaccine induced high levels of gp55-specific antibodies and conferred complete protection from lethal challenge with CSFV. Furthermore, all the vaccinated animals showed no adverse clinical signs of CSFV. In a subsequent study, 6-week-old weaned pigs and 7-day-old preweaned piglets were vaccinated with a DNA vaccine expressing the gp55/E2 gene from CSFV and then boosted with rPAV-gp55.[137] This prime–boost

vaccine approach induced high levels of gp55 antibody titers. Following challenge with CFSV, 100% of the weaned pigs and 75% of preweaned piglets were protected.

Similarly, a PAdV-3-based pseudorabies virus (PRV) vaccine encoding the glycoprotein D gene from a PRV strain was developed.[138] Vaccination of 5-week-old pigs with a single or two doses of the PAdV-3-PRV vaccine induced high levels of serum-neutralizing antibodies to PRV and conferred protection against challenge with a PRV virus. Pigs vaccinated with two doses of the PAdV-3-PRV vaccine had relatively higher PRV antibody titers and demonstrated better protection efficacy compared to those receiving a single vaccine dose.

A PAdV-3-based influenza vaccine (PAV3-HA) expressing HA of A/Hanoi/30408/2005 (H5N1) virus was evaluated for its potential to induce protective immunity in mice.[139] Vaccination of BALB/c mice with PAV3-HA induced high levels of HA-specific humoral and cellular responses. Vaccinated mice were protected against lethal challenge with a highly pathogenic avian H5N1 influenza virus. Interestingly, compared to a HAdV-5-based H5N1 vaccine, the immunity induced by PAV3-HA was present even twelve months postvaccination.

In addition to PAdV-3, PAdV-5 is also being investigated as a gene delivery tool. Tuboly and coworkers developed a recombinant PAdV-5 vector encoding the spike gene of transmissible gastroenteritis virus. Oral vaccination of pigs induced high levels of spike-specific antibodies.[140,141]

Like other AdV vectors, the prevalence of PAdV-neutralizing antibodies in the swine population is thought to hinder the use of PAdV vectors for vaccination purposes. A survey for PAdV-3 immunity in pigs from Australia demonstrated up to 90% prevalence of virus-neutralizing antibodies.[140] However, a study evaluating the performance of rPAV-gp55 in the presence of high levels of PAdV-3 antibodies demonstrated that the effect of rPAV-gp55 was not inhibited by the presence of elevated levels of PAdV-3-neutralizing antibodies.[135] Since preexisting HAdV-specific neutralizing antibodies in humans do not cross-neutralize PAdV-3,[133] PAdV-3 vectors would be a promising supplement to HAdV vectors as a delivery vehicle for recombinant vaccines and gene therapy applications in humans.

4.4 Ovine Adenovirus-Based Vectors

AdVs isolated from sheep are termed as ovine AdVs (OAdVs). Since 1969 seven serotypes of OAdVs have been isolated and classified (OAdV-1–7). OAdV-1 to 6 belong to the genus *Mastadenovirus*, while OAdV-7 belongs to the genus *Atadenovirus*. Most of these viruses are associated with either respiratory tract or intestinal tract infections. Among these serotypes, OAdV-7 has been well characterized and evaluated as a gene delivery vector. The nucleotide sequences of all seven OAdV serotypes are currently available. The genome organization of OAdV-7 is distinct from the other AdVs in the genus *Mastadenovirus* since its genome is AT rich.[142] Moreover, it lacks a clear distinguishable E1 region. The OAdV-7 entry into cells is independent of CAR and has in vivo tissue tropism distinct from HAdV-5.[143]

OAdV-7-based vectors were created using homologous recombination in *E. coli* and cosmid-based approaches followed by virus rescue in the ovine fetal lung cell

line (CSL503) or ovine fetal skin fibroblastic cell line.[142,144,145] Transgenes have been expressed from three unique sites, referred to as sites I, II, and III, in the OAdV-7 genome. Site I is located between the PVIII and fiber genes, site II is present within the RH2 gene approximately 902 bp from the 3′ end of the viral genome, and site III is located within a short noncoding region present between the E4 and the right-end transcription regions. Site III is considered the most stable among the three insertion sites, resulting in high levels of transgene expression independent of its orientation. Moreover, OAdV-7 vectors with site III insertions are easy to rescue and grow to high titers.[144]

An OAdV-7-based HCV vaccine (OAdV-NS3) encoding the nonstructural protein 3 (NS3) of the HCV BK strain has been developed.[146] The transgene was inserted at site III and was driven by the RSV 3′ LTR. Intramuscular immunization of BALB/c mice induced high levels of NS3-specific IFN-γ-secreting T-lymphocytes as measured by ELISpot assay. Interestingly, the NS3-specific T cell response persisted for up to 10 weeks postvaccination. Moreover, the OAdV-NS3-induced T cell response was not altered in the presence of immunity to HAdV-5.

In another study, an OAdV-based vector encoding protective recombinant antigen (45W) of *Taenia ovis* was generated and evaluated for its immunogenicity and protective efficacy in sheep when used alone or in combination with a DNA-based *T. ovis* vaccine.[147] Immunization of sheep with two doses of either OAdV or DNA vaccine induced low levels of 45W-specific antibody responses. However, immunization with the DNA vaccine followed by boosting with OAdV-based vaccine induced antibody responses >65-fold higher than those vaccinated with either the DNA or the OAdV vaccine alone, conferring protection from challenge with *T. ovis*.

An OAdV-7-based vaccine for HIV-1 (OAdV.HIVA) encoding the HIV-1 clade A consensus gag-derived protein coupled to a T cell polyepitope was developed.[148] Vaccination of mice with OAdV.HIVA either alone or in combination with HAdV-5 or MVA-vectored vaccines induced high levels of the HIV-1-specific T cell responses necessary to confer protection against HIV-1. This study demonstrated the potential of OAdV-7 as a delivery vehicle for HIV vaccines. Furthermore, the feasibility of using OAdV.HIVA in combination with BCG.HIVA(401) (a *Mycobacterium bovis* bacillus Calmette–Guérin (BCG)-based HIV-1) and MVA.HIVA was evaluated.[149] Unfortunately, vaccination with the BCG.HIVA(401) alone induced undetectable and weak CD8 T-cell responses in BALB/c mice and rhesus macaques, respectively. However, priming with the BCG.HIVA(401) followed by boosting with MVA.HIVA and OAdV.HIVA induced robust HIV-1-specific T-cell responses.

A recombinant OAdV-7 vector expressing the human alpha-1 antitrypsin gene (OAVhAAT) was generated and evaluated for its utility in human gene therapy in the skeletal muscle.[150] Injection of low doses of 3×10^7 infectious particles of OAVhAAT resulted in high serum levels of hAAT (>100 ng/ml), which was accompanied by a weak immune response to the vector. OAdV-7 infection was restricted to the smooth muscle with the level of hAAT expression comparable to that of a HAdV-5-based vector expressing the hAAT gene.[151]

Systemic administration of a single dose of an OAdV-7 vector encoding the *E. coli* purine fludarabine phosphorylase gene followed by prodrug fludarabine phosphate significantly inhibited the progression of prostate cancer in an immunocompetent

mouse model. The role of an OAdV-7-based vector expressing ovalbumin (OVA) was evaluated for inducing antitumor immunity in a mouse model.[152] Incubation of bone marrow-derived dendritic cells with the OAdV-7 vectors expressing OVA resulted in upregulation of costimulatory markers and production of IL-12. Splenocytes collected from the immunized animals actively responded to in vitro antigen stimulation. Furthermore, the in vivo cytotoxicity assays demonstrated efficient killing (up to 75%) of antigenic peptide-pulsed target cells. In mice inoculated with B16-OVA tumor cells, immunization with the OAdV7-OVA significantly suppressed tumor growth.

These studies highlight the potential of OAdV as a potential vehicle for gene delivery for vaccination and gene therapy purposes. Neutralizing antibodies against OAdV are not prevalent in humans, making OAdV vectors as promising tools for gene delivery in humans.[153]

4.5 Canine Adenovirus-Based Vectors

Canine AdV (CAdV) serotypes 1 and 2 have been well characterized. In dogs, CAdV-1 is responsible for infectious canine hepatitis, whereas CAdV-2 causes only mild upper respiratory tract infection. Despite the long history of cohabitation of dogs and humans, CAdVs are not able to cross the specific barriers and have not been associated with any human disease. The complete genome sequences of both CAdV-1[154] and CAdV-2[155] are available. Genome sizes of CAdV-1 and CAdV-2 are about 30.5 and 31.3 kb, respectively. Based on sequence analysis and genome organization, these CAdVs are classified under the genus *Mastadenovirus*.

An E3-deleted vector system based on CAdV-1 has been developed by homologous recombination in bacteria.[156] The E1-deleted CAdV-2 vectors were developed in the late 1990s and have an insertion capacity of ~4 kb.[157,158] The strategy employed for constructing CAdV-2 vectors involves homologous recombination between the viral genome and a shuttle vector carrying a transgene expression cassette along with other necessary sequences, and is similar to that used for generating HAdV vectors.[158,159] The E1-deleted CAdV-2 vectors cannot be *trans*-complemented by cell lines expressing human E1, necessitating the development of canine kidney cell lines expressing the CAdV-2 E1.[157,158] The E1-deleted CAdV-2 vectors can be grown to high titers (10^{13} vp/ml) and seem to have an excellent infectious particles-to-virus particles ratio.[158] However, the initial system for CAdV-2 vector development was cumbersome and very inefficient, often resulting in a CAdV-2 vector titer that was 10^4- to 10^5-fold lower than that of a HAdV-5 vector. Apparently this was because the canine kidney cells are difficult to transfect with linear CAdV-2 vector genomes of >30 kb.[157] Recently, an improved system for CAdV-2 vector generation has been described that uses a canine kidney cell line *trans*-complementing the CAdV-2 E1 and expressing I-*Sce*I fused to estrogen receptor.[160] This system allows highly efficient transfection of the supercoiled CAdV-2 vector genome into the canine kidney cells followed by an intracellular release of the vector genome and results in a 1000-fold increase in CAdV-2 vector titers.

A conditionally replicative AdV (CrAd) vector based on CAdV-2 has been developed and shows efficient replication and oncolytic potential in canine cell lines and a mouse xenograft model.[161] The subsequent study has demonstrated enhanced binding

and internalization of the CAdV-2 CrAd vector into canine osteosarcoma cells on vector modification by incorporation of polylysine into the C-terminus of fiber knobs.[162]

Tropism of CAdV-2 is distinct but overlaps with HAdV-5. Cells transduced by CAdV-2 can also be transduced by HAdV-5 but not vice versa.[163] CAdV-2 utilizes CAR as receptor but does not depend on $\alpha_M\beta_2$-integrins or the heavy chain of the MHC-I for entry. Interestingly, the CAdV-2 capsid lacks the RGD motif in the penton base required for interaction with integrins.[164–166] CAdV-2 also does not interact with other AdV receptors such as lactoferrin and CD46.[163] CAdV-2 is replication-defective for human cells, although it can infect human cells—a highly desirable trait for preventing any complication caused by a replication-competent AdV contamination.[157]

CAdV-2 vectors preferentially transduced rodent olfactory neurons and central nervous system (CNS) neurons in vitro and in vivo and demonstrated more efficient retrograde axonal transport than HAdV-5 following intramuscular and intrastriatum injections allowing for transgene expression throughout the substantia nigra.[167] Injection of a CAdV-2 vector into multiple sites into the striatum instead of a single site resulted in five times more dopaminergic neurons in the substantia nigra, suggesting that a multiple site injection strategy could prove more effective if a large area of the CNS is to be targeted.[168] CAdV-2 vectors are capable of transducing young mouse neurons without affecting the functional maturation of the neurons.[169] In addition, CAdV-2 vectors did not induce significant cellular infiltration in the rat brain, reflecting their poor immunogenicity in the CNS.[168] Collectively, these observations suggest that CAdV-2 vectors could prove to be a very effective tool for therapy of neurodegenerative diseases requiring widespread expression of transgene in the CNS without the complications arising from cellular infiltrations as a result of unintended immune activation. In addition to the CNS, the CAdV-2 vectors efficiently transduce mouse airway epithelial cells in vitro, ex vivo, and in vivo.[170]

Human sera containing high levels of HAdV-5-neutralizing antibodies demonstrated little to no detectable neutralization of CAdV-2.[157,158] In addition, sera from mice containing exceptionally high levels (titer 3360) of anti-HAdV-5-neutralizing antibodies did not affect transduction by CAdV-2 but caused greater than 97% inhibition of HAdV-5 in vitro. Similarly, the preexisting HAdV immunity did not significantly affect transduction of the murine respiratory tract by CAdV-2.[170] Collectively, these observations imply that the preexisting HAdV immunity does not affect CAdV-2 transduction and that CAdV-2 vectors are an effective tool for circumventing preexisting AdV immunity.

In addition to the E1-deleted, E3-deleted, and CrAdV vectors, helper-dependent (HD) vectors have also been developed based on CAdV-2.[168] The strategy for construction of HD CAdV-2 vectors involves homologous recombination in *E. coli* BJ5183 between the plasmid pEJK25 containing a CAdV-2 ITR (without the packaging sequences and~25 kb stuffer sequence) and the shuttle plasmid (pGut containing a transgene expression cassette, CAdV-2 ITR, with the packaging sequences, and a 2 kb overlap region with pEJK25).[168] The HD AdV vectors are generated by transfection of E1 complementing cell lines with the linearized HD vector genome followed by infection with the helper virus. Amplification of the HD vectors also requires coinfection with the helper virus. After several rounds of amplification, the HD vector is

purified by a cesium chloride density gradient.[171] Flanking the packaging domain of the helper virus with loxP sites does not prevent a significant level of contamination of the CAdV-2 vectors with the helper virus as the Cre-mediated excision at the loxP sites is not very efficient. However, mutations in *cis*-acting sequences in the packaging domain of the helper virus reduced the contamination to ~1% in HD CAdV-2 vector preparation with a final titer of 2.5×10^{10} infectious units/ml and $\sim 2 \times 10^{11}$ particles/ml. In addition, no replication-competent AdV could be detected in 10^{11} particles after multiple rounds of amplifications.

Importantly, the HD CAdV-2 vectors are capable of inducing long-term, sustained transgene expression in the rat brain lasting for at least one year postinjection.[168] An HD CAdV-2 vector also has induced transgene expression lasting up to 3 months postinstillation in the mouse upper respiratory tract. At 3 months, some decrease was detected compared to earlier time points, likely due to natural turnover of pulmonary epithelium. Additionally, the HD CAdV-2 vectors induced some degree of innate immune response in the mouse lung, but the level of induction was lower than that induced by HAdV-5 vectors.[170]

HD CAdV-2 vectors have also been tested for use in therapy of mucopolysaccharidosis (MPS) type VII which results from the deficiency of β-glucuronidase (β-glu) and is manifested as corneal clouding due to the accumulation of glycosaminoglycans (GAG).[172] The HD CAdV-2 vector encoding for human β-glu (HD-RIGIE) efficiently transduced CAR-positive keratocytes in mice and nonhuman primates following direct intrastromal injection. Apart from a temporary corneal edema that lasted for ~24 h, no major complication occurred. The CAdV-2 vector transduction was as efficient, if not more, as that of the HAdV-5 vector. It covered the entire cornea but declined after 1 week, suggesting that further improvements are necessary to obtain long-term expression. Interestingly, the CAdV-2 vector induced a histological correction of GAG and cell morphology in the canine cornea, possibly due to the CAR expression by keratocytes and a high-level expression of β-glu by the vector.[172] In addition, the HD-RIGIE could also reverse neuropathological changes associated with MPS VII in the dog's brain.[173] This HD-RIGIE has the potential to induce long-term β-glu expression in mice, resulting in decreased GAG levels, lysosomal enzyme activity, and most importantly, a dramatic improvement in cognitive function.[174] For these reasons, HD CAdV-2 vectors can potentially provide tools for therapy of MPS VII and other lysosomal storage diseases.

4.6 Murine Adenovirus-Based Vectors

The murine AdVs are classified under the genus *Mastadenovirus*—species A, B, and C. The murine AdV serotype 1 (MAdV-1) was first isolated and characterized in 1960[175] and has been used as a model system for exploring virus–host interactions, AdV pathogenesis, and antiviral therapies.[176] Infection with MAdV-1 can cause serious disease in both newborn and adult mice. MAdV-1 infections, even at low doses, in newborn mice can cause serious mortality. However, akin to HAdVs, MAdV-1 infections in immunocompetent adult mice cause only mild infections with low mortality.[177,178] Unlike HAdVs, which initially infect the respiratory epithelium, MAdV-1

replicates in the primary endothelial cells of various body systems and thus results in widespread systemic infection affecting the liver, spleen, kidneys, intestines, adrenals, heart, brain, and spinal cord.[179,180]

The genome of MAdV-1 is ~30.9 kbp and is similar to HAdV-5 in organization except that it does not encode virus-associated RNAs.[181,182] Vectors based on MAdV-1 were first described in the late 1990s.[181,183,184] E1A-deleted MAdV-1 vectors are constructed either by replacing an initiation codon with a stop codon or by deleting each of the three conserved regions CR1, CR2, or CR3 within E1A. These vectors grew to titers only one log lower than those of wild-type virus in mouse fibroblasts, implying that MAdV-1 E1A is not essential for efficient virus replication.[184] Deletion of E1A did not alter the gene expression levels of the other early region genes, suggesting that, unlike HAdVs, the MAdV-1 E1A is not required for transactivation of other early region genes.[184] However, the pathogenicity of E1A null mutants of MAdV-1 is significantly lower than wild-type virus, suggesting the requirement of E1A in the host but not in the cell culture.[179]

The E3 region of MAdV-1 encodes for three proteins with a common N-terminal sequence but unique C-terminal sequences.[183] E3-deleted MAdV-1 vectors were constructed by mutagenesis to block expression of each one of the E3 proteins.[181,185] The E3 mutants of MAdV-1 induced significantly less endothelial cell damage and inflammatory response in the brain and spinal cord than the wild-type virus, indicating that there is a role for E3 proteins in these areas.[181] The MAdV-1 does not utilize CAR as a primary receptor[176] and does not contain the RGD motif in the penton base.[186] Instead, the MAdV-1 fiber knob domain carries an RGD motif that plays an important role in MAdV-1 entry mediated by αV-integrins. In addition, cell surface glycosaminoglycan heparan sulfate is also involved in MAdV-1 infection.[186,187] MAdV-1 binds to primary human smooth muscles with significantly higher affinity than HAdV-5. The biodistribution of MAdV-1 is not altered by the presence of physiological concentrations of coagulation factor XI or the vitamin K-dependent factors that play a role in the targeting of HAdV-5 to the liver. Although MAdV-1 does bind to the factor XI, contrary to HAdV-5, the binding does not result in cell attachment. Consequently, the targeting of MAdV-1 to the liver is significantly lower than for HAdV-5.[188]

A MAdV-1 vector carrying a deletion in the CR2 region of E1A (dlE102) has been shown to be an excellent oncolytic AdV system that can help in understanding the mechanism of the action of oncolytic AdVs in an immunocompetent host.[189] The ability to replicate in an immunocompetent host is particularly noteworthy as most of the oncolytic AdV vectors based on HAdV-5 cannot replicate in mouse tissue, and, therefore, their safety and efficacy cannot be adequately evaluated. The dlE102 virus replicates efficiently in murine tumor cells but its replication is attenuated in nontransformed cells. In addition, it demonstrates potent antitumor activity in an immunocompetent xenograft tumor model.[189] A feasible approach to arm the dlE102 virus with an immunomodulatory molecule has further enhanced its antitumor efficacy.[189] MAdV-1 has also been used as a model for understanding the pathogenic mechanisms of pediatric myocarditis[190,191] and acute respiratory infection[192] caused by AdVs.

4.7 Avian Adenovirus-Based Vectors

Several AdVs have been isolated from birds including fowl (FAdV), falcon (FaAdV), goose (GoAdV), ducks (DAdV), and turkey (TAdV). Most of these viruses are included under the genus *Aviadenovirus* and grouped in to four serotypes (FaAdVs A–E, GoAdV A, DAdV A, and TAdV B*).* Duck AdV A, AdV 127, and egg drop syndrome 76 virus, which is also known as duck AdV 1 (DAdV-1), are included under the genus *Atadenovirus* based on their unique genomic and structural characteristics. These viruses are serologically distinct from other AdV genera and infect only birds.

The complete nucleotide sequences of five *Aviadenovirus* species (FAdV-1, FAdV-4, FAdV-8, FAdV-9, and TAdV-1) have been determined.[193,194] The genomes of aviadenoviruses are 20–45% larger than other AdVs and range in size between 43,804 and 45,667 bp. Furthermore, the genome organization of aviadenoviruses is distinct compared with other AdVs and contains a high G+C content (ranging from 50% to 67%). The virions from FAdV-1, FAdV-4, and TAdV-1 contain two fibers per penton base[195] while genomes of aviadenoviruses lack the genes encoding E1, E3, V, and IX proteins. The aviadenovirus genomes contain several uncharacterized transcription units at the right end that are unique to this genus.[196]

FAdV-based vectors devoid of GAM-1 have been created using homologous recombination in *E. coli* with cosmid-based approaches followed by transfection in permissive Leghorn male hepatoma cells. Three aviadenoviruses (FAdV-1, FAdV-8, and FAdV-10) have been evaluated as gene delivery vectors for vaccination. A FAdV-10 vector containing the VP2 gene from the Australian classical strain 002/73 of infectious bursal disease virus (IBDV) has been generated.[197] Vaccination of specific pathogen-free chickens with this FAdV10-based vaccine induced VP2-specific antibodies that protected chickens against challenge with the IBDV V877 strain. A FAdV-1-based vector has been evaluated for cancer gene therapy and demonstrated efficient transduction of several human cells including HepG2, A549, and primary human dermal fibroblasts.[198] In another study, a FAdV-8-based vaccine was developed against infectious bronchitis virus (IBV) after incorporating the spike peplomer S1 subunit from the IBV Vic S strain under the control of the FAdV major late promoter.[199] Using this FAdV-8 vector-based vaccine, immunization of commercial broiler chickens induced S1-specific antibody responses and conferred protection against challenge with either Vic S (serotype B) or N1/62 (serotype C) strains of IBV. A FAdV-8 vector expressing cytokines including chicken IFN-γ has been developed and evaluated to enhance the immunogenicity of vaccines in chickens.[200]

5. Concluding Remarks

A number of nonhuman AdVs including simian (SAdV), bovine (BAdV), porcine (PAdV), canine (CAdV), ovine (OAdV), murine (MAdV, and fowl (FAdV) are at various stages of development as gene delivery systems for recombinant vaccines and gene therapy applications. In addition to effectively circumventing preexisting HAdV immunity, these nonhuman AdV vectors can utilize a number of other receptors in

addition to CAR for vector internalization, thereby expanding the range of cell types that can be targeted. The safety aspects of these vectors appear to be similar to or better than HAdV vectors. In addition to their utility for human applications, nonhuman AdV vectors also provide excellent platforms for veterinary vaccines. A specific nonhuman AdV vector when used in its species of origin could provide an excellent animal model for evaluating the efficacy and pathogenesis of these vectors.

The mechanism/s of activation of innate immunity including TLR expression by nonhuman AdVs needs to be determined to fully explore their potential as gene delivery systems. These vectors will be useful in prime–boost approaches with other AdV vectors or with other gene delivery systems including DNA immunization or other viral or bacterial vectors. In situations where multiple vector inoculations are required for a desired effect, nonhuman AdV vectors could supplement HAdV or other viral vectors. Only SAdV vectors can be grown in certified human cell lines that are used for HAdV replication and purification; therefore, there is a need to certify a number of other cell lines that are suitable for growing and purifying other nonhuman AdV vectors. To fully exploit the desired impact of using nonhuman AdVs, further changes in nonhuman AdV vector design will be necessary.

Acknowledgments

This work was supported by Hatch Fund and we are thankful to Jane Kovach for her excellent secretarial assistance.

References

1. Bonnet MC, Tartaglia J, Verdier F, Kourilsky P, Lindberg A, Klein M, et al. Recombinant viruses as a tool for therapeutic vaccination against human cancers. *Immunol Lett* 2000;**74**:11–25.
2. Souza AP, Haut L, Reyes-Sandoval A, Pinto AR. Recombinant viruses as vaccines against viral diseases. *Braz J Med Biol Res* 2005;**38**:509–22.
3. Rocha CD, Caetano BC, Machado AV, Bruna-Romero O. Recombinant viruses as tools to induce protective cellular immunity against infectious diseases. *Int Microbiol* 2004;**7**:83–94.
4. Barouch DH. Rational design of gene-based vaccines. *J Pathol* 2006;**208**:283–9.
5. Rowe WP, Huebner RJ, Gilmore LK, Parrot RH, Ward TG. Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. *Proc Soc Exp Biol Med* 1953;**84**:570–3.
6. Huebner RJ, Rowe WP, Schatten WE, Smith RR, Thomas LB. Studies on the use of viruses in the treatment of carcinoma of the cervix. *Cancer* 1956;**9**:1211–8.
7. Russell WC. Adenoviruses: update on structure and function. *J Gen Virol* 2009;**90**:1–20.
8. Harrach B, Benko M, Both GW, Brown M, Davison AJ, Echavarria M, et al. The double stranded DNA viruses: adenoviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. *Virus taxonomy: ninth report of the international committee on taxonomy of viruses*. 1st ed Waltham (MA): Academic Press: Elsevier; 2011. p. 125–41.

9. Davison AJ, Benko M, Harrach B. Genetic content and evolution of adenoviruses. *J Gen Virol* 2003;**84**:2895–908.
10. Sharma A, Li X, Bangari DS, Mittal SK. Adenovirus receptors and their implications in gene delivery. *Virus Res* 2009;**143**:184–94.
11. Arnberg N. Adenovirus receptors: implications for targeting of viral vectors. *Trends Pharmacol Sci* 2012;**33**:442–8.
12. Bangari DS, Mittal SK. Current strategies and future directions for eluding adenoviral vector immunity. *Curr Gene Ther* 2006;**6**:215–26.
13. Sakurai F, Kawabata K, Mizuguchi H. Adenovirus vectors composed of subgroup B adenoviruses. *Curr Gene Ther* 2007;**7**:229–38.
14. Russell WC. Update on adenovirus and its vectors. *J Gen Virol* 2000;**81**:2573–604.
15. Edelstein ML, Abedi MR, Wixon J, Edelstein RM. Gene therapy clinical trials worldwide 1989–2004-an overview. *J Gene Med* 2004;**6**:597–602.
16. Mast TC, Kierstead L, Gupta SB, Nikas AA, Kallas EG, Novitsky V, et al. International epidemiology of human pre-existing adenovirus (Ad) type-5, type-6, type-26 and type-36 neutralizing antibodies: correlates of high Ad5 titers and implications for potential HIV vaccine trials. *Vaccine* 2010;**28**:950–7.
17. Yu B, Zhou Y, Wu H, Wang Z, Zhan Y, Feng X, et al. Seroprevalence of neutralizing antibodies to human adenovirus type 5 in healthy adults in China. *J Med Virol* 2012;**84**:1408–14.
18. Sumida SM, Truitt DM, Lemckert AA, Vogels R, Custers JH, Addo MM, et al. Neutralizing antibodies to adenovirus serotype 5 vaccine vectors are directed primarily against the adenovirus hexon protein. *J Immunol* 2005;**174**:7179–85.
19. Barouch DH, Kik SV, Weverling GJ, Dilan R, King SL, Maxfield LF, et al. International seroepidemiology of adenovirus serotypes 5, 26, 35, and 48 in pediatric and adult populations. *Vaccine* 2011;**29**:5203–9.
20. Chirmule N, Propert K, Magosin S, Qian Y, Qian R, Wilson J. Immune responses to adenovirus and adeno-associated virus in humans. *Gene Ther* 1999;**6**:1574–83.
21. Gahery-Segard H, Juillard V, Gaston J, Lengagne R, Pavirani A, Boulanger P, et al. Humoral immune response to the capsid components of recombinant adenoviruses: routes of immunization modulate virus-induced Ig subclass shifts. *Eur J Immunol* 1997;**27**:653–9.
22. Gahery-Segard H, Farace F, Godfrin D, Gaston J, Lengagne R, Tursz T, et al. Immune response to recombinant capsid proteins of adenovirus in humans: antifiber and anti-penton base antibodies have a synergistic effect on neutralizing activity. *J Virol* 1998;**72**:2388–97.
23. Bruder JT, Semenova E, Chen P, Limbach K, Patterson NB, Stefaniak ME, et al. Modification of Ad5 hexon hypervariable regions circumvents pre-existing Ad5 neutralizing antibodies and induces protective immune responses. *PLoS One* 2012;**7**:e33920.
24. Mittal SK, McDermott MR, Johnson DC, Prevec L, Graham FL. Monitoring foreign gene expression by a human adenovirus-based vector using the firefly luciferase gene as a reporter. *Virus Res* 1993;**28**:67–90.
25. Chirmule N, Raper SE, Burkly L, Thomas D, Tazelaar J, Hughes JV, et al. Readministration of adenovirus vector in nonhuman primate lungs by blockade of CD40-CD40 ligand interactions. *J Virol* 2000;**74**:3345–52.
26. Moffatt S, Hays J, HogenEsch H, Mittal SK. Circumvention of vector-specific neutralizing antibody response by alternating use of human and non-human adenoviruses: implications in gene therapy. *Virology* 2000;**272**:159–67.
27. Pichla-Gollon SL, Lin SW, Hensley SE, Lasaro MO, Herkenhoff-Haut L, Drinker M, et al. Effect of preexisting immunity on an adenovirus vaccine vector: in vitro neutralization assays fail to predict inhibition by antiviral antibody in vivo. *J Virol* 2009;**83**:5567–73.

28. Fausther-Bovendo H, Kobinger GP. Pre-existing immunity against Ad vectors: humoral, cellular and innate response, what's important? *Hum Vaccin Immunother* 2014;**10**:2875–84.
29. Olive M, Eisenlohr L, Flomenberg N, Hsu S, Flomenberg P. The adenovirus capsid protein hexon contains a highly conserved human CD4+ T-cell epitope. *Hum Gene Ther* 2002;**13**:1167–78.
30. Leen AM, Sili U, Vanin EF, Jewell AM, Xie W, Vignali D, et al. Conserved CTL epitopes on the adenovirus hexon protein expand subgroup cross-reactive and subgroup-specific CD8+ T cells. *Blood* 2004;**104**:2432–40.
31. Tang J, Olive M, Pulmanausahakul R, Schnell M, Flomenberg N, Eisenlohr L, et al. Human CD8+ cytotoxic T cell responses to adenovirus capsid proteins. *Virology* 2006;**350**:312–22.
32. Heemskerk B, Veltrop-Duits LA, van VT, ten Dam MM, Heidt S, Toes RE, et al. Extensive cross-reactivity of CD4+ adenovirus-specific T cells: implications for immunotherapy and gene therapy. *J Virol* 2003;**77**:6562–6.
33. Hutnick NA, Carnathan D, Demers K, Makedonas G, Ertl HC, Betts MR. Adenovirus-specific human T cells are pervasive, polyfunctional, and cross-reactive. *Vaccine* 2010;**28**:1932–41.
34. Veltrop-Duits LA, Heemskerk B, Sombroek CC, van VT, Gubbels S, Toes RE, et al. Human CD4+ T cells stimulated by conserved adenovirus 5 hexon peptides recognize cells infected with different species of human adenovirus. *Eur J Immunol* 2006;**36**:2410–23.
35. Calcedo R, Vandenberghe LH, Roy S, Somanathan S, Wang L, Wilson JM. Host immune responses to chronic adenovirus infections in human and nonhuman primates. *J Virol* 2009;**83**:2623–31.
36. Schnell MA, Zhang Y, Tazelaar J, Gao GP, Yu QC, Qian R, et al. Activation of innate immunity in nonhuman primates following intraportal administration of adenoviral vectors. *Mol Ther* 2001;**3**:708–22.
37. Zhang Y, Chirmule N, Gao GP, Qian R, Croyle M, Joshi B, et al. Acute cytokine response to systemic adenoviral vectors in mice is mediated by dendritic cells and macrophages. *Mol Ther* 2001;**3**:697–707.
38. Raper SE, Chirmule N, Lee FS, Wivel NA, Bagg A, Gao GP, et al. Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. *Mol Genet Metab* 2003;**80**:148–58.
39. Basner-Tschakarjan E, Gaffal E, O'Keeffe M, Tormo D, Limmer A, Wagner H, et al. Adenovirus efficiently transduces plasmacytoid dendritic cells resulting in TLR9-dependent maturation and IFN-alpha production. *J Gene Med* 2006;**8**:1300–6.
40. Thaci B, Ulasov IV, Wainwright DA, Lesniak MS. The challenge for gene therapy: innate immune response to adenoviruses. *Oncotarget* 2011;**2**:113–21.
41. Chen RF, Lee CY. Adenoviruses types, cell receptors and local innate cytokines in adenovirus infection. *Int Rev Immunol* 2014;**33**:45–53.
42. Hendrickx R, Stichling N, Koelen J, Kuryk L, Lipiec A, Greber UF. Innate immunity to adenovirus. *Hum Gene Ther* 2014;**25**:265–84.
43. Huang X, Yang Y. Innate immune recognition of viruses and viral vectors. *Hum Gene Ther* 2009;**20**:293–301.
44. Fejer G, Freudenberg M, Greber UF, Gyory I. Adenovirus-triggered innate signalling pathways. *Eur J Microbiol Immunol (Bp)* 2011;**1**:279–88.
45. Lopez-Gordo E, Podgorski II, Downes N, Alemany R. Circumventing antivector immunity: potential use of nonhuman adenoviral vectors. *Hum Gene Ther* 2014;**25**:285–300.
46. Capasso C, Garofalo M, Hirvinen M, Cerullo V. The evolution of adenoviral vectors through genetic and chemical surface modifications. *Viruses* 2014;**6**:832–55.

47. Ahi YS, Bangari DS, Mittal SK. Adenoviral vector immunity: its implications and circumvention strategies. *Curr Gene Ther* 2011;**11**:307–20.
48. Bangari DS, Mittal SK. Development of nonhuman adenoviruses as vaccine vectors. *Vaccine* 2006;**24**:849–62.
49. Farina SF, Gao GP, Xiang ZQ, Rux JJ, Burnett RM, Alvira MR, et al. Replication-defective vector based on a chimpanzee adenovirus. *J Virol* 2001;**75**:11603–13.
50. Crawford-Miksza L, Schnurr DP. Analysis of 15 adenovirus hexon proteins reveals the location and structure of seven hypervariable regions containing serotype-specific residues. *J Virol* 1996;**70**:1836–44.
51. Cohen CJ, Xiang ZQ, Gao GP, Ertl HC, Wilson JM, Bergelson JM. Chimpanzee adenovirus CV-68 adapted as a gene delivery vector interacts with the coxsackievirus and adenovirus receptor. *J Gen Virol* 2002;**83**:151–5.
52. Roy S, Gao G, Clawson DS, Vandenberghe LH, Farina SF, Wilson JM. Complete nucleotide sequences and genome organization of four chimpanzee adenoviruses. *Virology* 2004;**324**:361–72.
53. Tatsis N, Blejer A, Lasaro MO, Hensley SE, Cun A, Tesema L, et al. A CD46-binding chimpanzee adenovirus vector as a vaccine carrier. *Mol Ther* 2007;**15**:608–17.
54. Tatsis N, Tesema L, Robinson ER, Giles-Davis W, McCoy K, Gao GP, et al. Chimpanzee-origin adenovirus vectors as vaccine carriers. *Gene Ther* 2006;**13**:421–9.
55. Chen H, Smith GJ, Li KS, Wang J, Fan XH, Rayner JM, et al. Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. *Proc Natl Acad Sci USA* 2006;**103**:2845–50.
56. Ersching J, Hernandez MI, Cezarotto FS, Ferreira JD, Martins AB, Switzer WM, et al. Neutralizing antibodies to human and simian adenoviruses in humans and New-World monkeys. *Virology* 2010;**407**:1–6.
57. Colloca S, Barnes E, Folgori A, Ammendola V, Capone S, Cirillo A, et al. Vaccine vectors derived from a large collection of simian adenoviruses induce potent cellular immunity across multiple species. *Sci Transl Med* 2012;**4**:115ra2.
58. Xiang Z, Li Y, Cun A, Yang W, Ellenberg S, Switzer WM, et al. Chimpanzee adenovirus antibodies in humans, sub-Saharan Africa. *Emerg Infect Dis* 2006;**12**:1596–9.
59. Zhang S, Huang W, Zhou X, Zhao Q, Wang Q, Jia B. Seroprevalence of neutralizing antibodies to human adenoviruses type-5 and type-26 and chimpanzee adenovirus type-68 in healthy Chinese adults. *J Med Virol* 2013;**85**:1077–84.
60. Jian L, Zhao Q, Zhang S, Huang W, Xiong Y, Zhou X, et al. The prevalence of neutralising antibodies to chimpanzee adenovirus type 6 and type 7 in healthy adult volunteers, patients with chronic hepatitis B and patients with primary hepatocellular carcinoma in China. *Arch Virol* 2014;**159**:465–70.
61. Dudareva M, Andrews L, Gilbert SC, Bejon P, Marsh K, Mwacharo J, et al. Prevalence of serum neutralizing antibodies against chimpanzee adenovirus 63 and human adenovirus 5 in Kenyan children, in the context of vaccine vector efficacy. *Vaccine* 2009;**27**:3501–4.
62. Chen H, Xiang ZQ, Li Y, Kurupati RK, Jia B, Bian A, et al. Adenovirus-based vaccines: comparison of vectors from three species of adenoviridae. *J Virol* 2010;**84**:10522–32.
63. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet* 2008;**372**:1881–93.
64. Ewer KJ, O'Hara GA, Duncan CJ, Collins KA, Sheehy SH, Reyes-Sandoval A, et al. Protective $CD8^+$ T-cell immunity to human malaria induced by chimpanzee adenovirus-MVA immunisation. *Nat Commun* 2013;**4**:2836.

65. Abbink P, Lemckert AA, Ewald BA, Lynch DM, Denholtz M, Smits S, et al. Comparative seroprevalence and immunogenicity of six rare serotype recombinant adenovirus vaccine vectors from subgroups B and D. *J Virol* 2007;**81**:4654–63.
66. Kahl CA, Bonnell J, Hiriyanna S, Fultz M, Nyberg-Hoffman C, Chen P, et al. Potent immune responses and in vitro pro-inflammatory cytokine suppression by a novel adenovirus vaccine vector based on rare human serotype 28. *Vaccine* 2010;**28**:5691–702.
67. Quinn KM, Da CA, Yamamoto A, Berry D, Lindsay RW, Darrah PA, et al. Comparative analysis of the magnitude, quality, phenotype, and protective capacity of simian immunodeficiency virus gag-specific $CD8^+$ T cells following human-, simian-, and chimpanzee-derived recombinant adenoviral vector immunization. *J Immunol* 2013;**190**:2720–35.
68. Haut LH, Lin SW, Tatsis N, DiMenna LJ, Giles-Davis W, Pinto AR, et al. Robust genital gag-specific $CD8^+$ T-cell responses in mice upon intramuscular immunization with simian adenoviral vectors expressing HIV-1-gag. *Eur J Immunol* 2010;**40**:3426–38.
69. Peruzzi D, Dharmapuri S, Cirillo A, Bruni BE, Nicosia A, Cortese R, et al. A novel chimpanzee serotype-based adenoviral vector as delivery tool for cancer vaccines. *Vaccine* 2009;**27**:1293–300.
70. Skog J, Edlund K, Bergenheim AT, Wadell G. Adenoviruses 16 and CV23 efficiently transduce human low-passage brain tumor and cancer stem cells. *Mol Ther* 2007;**15**:2140–5.
71. Wang D, Raja NU, Trubey CM, Juompan LY, Luo M, Woraratanadharm J, et al. Development of a cAdVax-based bivalent ebola virus vaccine that induces immune responses against both the Sudan and Zaire species of Ebola virus. *J Virol* 2006;**80**:2738–46.
72. Kobinger GP, Feldmann H, Zhi Y, Schumer G, Gao G, Feldmann F, et al. Chimpanzee adenovirus vaccine protects against Zaire Ebola virus. *Virology* 2006;**346**:394–401.
73. Roy S, Zhi Y, Kobinger GP, Figueredo J, Calcedo R, Miller JR, et al. Generation of an adenoviral vaccine vector based on simian adenovirus 21. *J Gen Virol* 2006;**87**:2477–85.
74. Stanley DA, Honko AN, Asiedu C, Trefry JC, Lau-Kilby AW, Johnson JC, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. *Nat Med* 2014;**20**:1126–9.
75. Sharma A, Wendland R, Sung B, Wu W, Grunwald T, Worgall S. Maternal immunization with chimpanzee adenovirus expressing RSV fusion protein protects against neonatal RSV pulmonary infection. *Vaccine* 2014;**32**:5761–8.
76. Xiang ZQ, Greenberg L, Ertl HC, Rupprecht CE. Protection of non-human primates against rabies with an adenovirus recombinant vaccine. *Virology* 2014;**450-451**:243–9.
77. Zhou D, Cun A, Li Y, Xiang Z, Ertl HC. A chimpanzee-origin adenovirus vector expressing the rabies virus glycoprotein as an oral vaccine against inhalation infection with rabies virus. *Mol Ther* 2006;**14**:662–72.
78. Roy S, Kobinger GP, Lin J, Figueredo J, Calcedo R, Kobasa D, et al. Partial protection against H5N1 influenza in mice with a single dose of a chimpanzee adenovirus vector expressing nucleoprotein. *Vaccine* 2007;**25**:6845–51.
79. Vitelli A, Quirion MR, Lo CY, Misplon JA, Grabowska AK, Pierantoni A, et al. Vaccination to conserved influenza antigens in mice using a novel simian adenovirus vector, PanAd3, derived from the bonobo *Pan paniscus*. *PLoS One* 2013;**8**:e55435.
80. Reyes-Sandoval A, Sridhar S, Berthoud T, Moore AC, Harty JT, Gilbert SC, et al. Single-dose immunogenicity and protective efficacy of simian adenoviral vectors against *Plasmodium berghei*. *Eur J Immunol* 2008;**38**:732–41.
81. Sridhar S, Reyes-Sandoval A, Draper SJ, Moore AC, Gilbert SC, Gao GP, et al. Single-dose protection against *Plasmodium berghei* by a simian adenovirus vector using a human cytomegalovirus promoter containing intron A. *J Virol* 2008;**82**:3822–33.

82. Warimwe GM, Lorenzo G, Lopez-Gil E, Reyes-Sandoval A, Cottingham MG, Spencer AJ, et al. Immunogenicity and efficacy of a chimpanzee adenovirus-vectored Rift Valley fever vaccine in mice. *Virol J* 2013;**10**:349.
83. Kobinger GP, Figueredo JM, Rowe T, Zhi Y, Gao G, Sanmiguel JC, et al. Adenovirus-based vaccine prevents pneumonia in ferrets challenged with the SARS coronavirus and stimulates robust immune responses in macaques. *Vaccine* 2007;**25**:5220–31.
84. Borthwick N, Ahmed T, Ondondo B, Hayes P, Rose A, Ebrahimsa U, et al. Vaccine-elicited human T cells recognizing conserved protein regions inhibit HIV-1. *Mol Ther* 2014;**22**:464–75.
85. Altfeld M, Addo MM, Rosenberg ES, Hecht FM, Lee PK, Vogel M, et al. Influence of HLA-B57 on clinical presentation and viral control during acute HIV-1 infection. *Aids* 2003;**17**:2581–91.
86. Ferguson AL, Mann JK, Omarjee S, Ndung'u T, Walker BD, Chakraborty AK. Translating HIV sequences into quantitative fitness landscapes predicts viral vulnerabilities for rational immunogen design. *Immunity* 2013;**38**:606–17.
87. Kelleher AD, Long C, Holmes EC, Allen RL, Wilson J, Conlon C, et al. Clustered mutations in HIV-1 gag are consistently required for escape from HLA-B27-restricted cytotoxic T lymphocyte responses. *J Exp Med* 2001;**193**:375–86.
88. Leslie AJ, Pfafferott KJ, Chetty P, Draenert R, Addo MM, Feeney M, et al. HIV evolution: CTL escape mutation and reversion after transmission. *Nat Med* 2004;**10**:282–9.
89. Hayton EJ, Rose A, Ibrahimsa U, Del SM, Capone S, Crook A, et al. Safety and tolerability of conserved region vaccines vectored by plasmid DNA, simian adenovirus and modified vaccinia virus ankara administered to human immunodeficiency virus type 1-uninfected adults in a randomized, single-blind phase I trial. *PLoS One* 2014;**9**:e101591.
90. Hoffman SL, Isenbarger D, Long GW, Sedegah M, Szarfman A, Waters L, et al. Sporozoite vaccine induces genetically restricted T cell elimination of malaria from hepatocytes. *Science* 1989;**244**:1078–81.
91. Khusmith S, Sedegah M, Hoffman SL. Complete protection against *Plasmodium yoelii* by adoptive transfer of a $CD8^+$ cytotoxic T-cell clone recognizing sporozoite surface protein 2. *Infect Immun* 1994;**62**:2979–83.
92. Romero P, Maryanski JL, Corradin G, Nussenzweig RS, Nussenzweig V, Zavala F. Cloned cytotoxic T cells recognize an epitope in the circumsporozoite protein and protect against malaria. *Nature* 1989;**341**:323–6.
93. Schofield L, Villaquiran J, Ferreira A, Schellekens H, Nussenzweig R, Nussenzweig V. Gamma interferon, $CD8^+$ T cells and antibodies required for immunity to malaria sporozoites. *Nature* 1987;**330**:664–6.
94. Weiss WR, Mellouk S, Houghten RA, Sedegah M, Kumar S, Good MF, et al. Cytotoxic T cells recognize a peptide from the circumsporozoite protein on malaria-infected hepatocytes. *J Exp Med* 1990;**171**:763–73.
95. Hill AV, Reyes-Sandoval A, O'Hara G, Ewer K, Lawrie A, Goodman A, et al. Prime-boost vectored malaria vaccines: progress and prospects. *Hum Vaccin* 2010;**6**:78–83.
96. Ogwang C, Afolabi M, Kimani D, Jagne YJ, Sheehy SH, Bliss CM, et al. Safety and immunogenicity of heterologous prime-boost immunisation with *Plasmodium falciparum* malaria candidate vaccines, ChAd63 ME-TRAP and MVA ME-TRAP, in healthy Gambian and Kenyan adults. *PLoS One* 2013;**8**:e57726.
97. Sheehy SH, Duncan CJ, Elias SC, Biswas S, Collins KA, O'Hara GA, et al. Phase Ia clinical evaluation of the safety and immunogenicity of the *Plasmodium falciparum* blood-stage antigen AMA1 in ChAd63 and MVA vaccine vectors. *PLoS One* 2012;**7**:e31208.

98. Sheehy SH, Duncan CJ, Elias SC, Collins KA, Ewer KJ, Spencer AJ, et al. Phase Ia clinical evaluation of the *Plasmodium falciparum* blood-stage antigen MSP1 in ChAd63 and MVA vaccine vectors. *Mol Ther* 2011;**19**:2269–76.
99. O'Hara GA, Duncan CJ, Ewer KJ, Collins KA, Elias SC, Halstead FD, et al. Clinical assessment of a recombinant simian adenovirus ChAd63: a potent new vaccine vector. *J Infect Dis* 2012;**205**:772–81.
100. Hayes CN, Chayama K. Emerging treatments for chronic hepatitis C. *J Formos Med Assoc* 2015;**114**:204–15.
101. Shoukry NH, Grakoui A, Houghton M, Chien DY, Ghrayeb J, Reimann KA, et al. Memory $CD8^+$ T cells are required for protection from persistent hepatitis C virus infection. *J Exp Med* 2003;**197**:1645–55.
102. Urbani S, Amadei B, Fisicaro P, Tola D, Orlandini A, Sacchelli L, et al. Outcome of acute hepatitis C is related to virus-specific CD4 function and maturation of antiviral memory CD8 responses. *Hepatology* 2006;**44**:126–39.
103. Folgori A, Capone S, Ruggeri L, Meola A, Sporeno E, Ercole BB, et al. A T-cell HCV vaccine eliciting effective immunity against heterologous virus challenge in chimpanzees. *Nat Med* 2006;**12**:190–7.
104. Barnes E, Folgori A, Capone S, Swadling L, Aston S, Kurioka A, et al. Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man. *Sci Transl Med* 2012;**4**:115ra1.
105. Reddy PS, Idamakanti N, Zakhartchouk AN, Baxi MK, Lee JB, Pyne C, et al. Nucleotide sequence, genome organization, and transcription map of bovine adenovirus type 3. *J Virol* 1998;**72**:1394–402.
106. Mittal SK, Prevec L, Babiuk LA, Graham FL. Sequence analysis of bovine adenovirus type 3 early region 3 and fibre protein genes. *J Gen Virol* 1992;**73**:3295–300.
107. Zheng B, Mittal SK, Graham FL, Prevec L. The E1 sequence of bovine adenovirus type 3 and complementation of human adenovirus type 5 E1A function in bovine cells. *Virus Res* 1994;**31**:163–86.
108. Bangari DS, Shukla S, Mittal SK. Comparative transduction efficiencies of human and nonhuman adenoviral vectors in human, murine, bovine, and porcine cells in culture. *Biochem Biophys Res Commun* 2005;**327**:960–6.
109. Ruigrok RW, Barge A, Mittal SK, Jacrot B. The fibre of bovine adenovirus type 3 is very long but bent. *J Gen Virol* 1994;**75**:2069–73.
110. Mittal SK, Middleton DM, Tikoo SK, Babiuk LA. Pathogenesis and immunogenicity of bovine adenovirus type 3 in cotton rats (*Sigmodon hispidus*). *Virology* 1995;**213**:131–9.
111. Mittal SK, Middleton DM, Tikoo SK, Prevec L, Graham FL, Babiuk LA. Pathology and immunogenicity in the cotton rat (*Sigmodon hispidus*) model after infection with a bovine adenovirus type 3 recombinant virus expressing the firefly luciferase gene. *J Gen Virol* 1996;**77**:1–9.
112. Mittal SK, Tikoo SK, Van Donkersgoed J, Beskorwayne T, Godson DL, Babiuk LA. Experimental inoculation of heifers with bovine adenovirus type 3. *Can J Vet Res* 1999; **63**:153–6.
113. Mittal SK, Prevec L, Graham FL, Babiuk LA. Development of a bovine adenovirus type 3-based expression vector. *J Gen Virol* 1995;**76**:93–102.
114. van Olphen AL, Mittal SK. Generation of infectious genome of bovine adenovirus type 3 by homologous recombination in bacteria. *J Virol Methods* 1999;**77**:125–9.
115. Reddy PS, Idamakanti N, Chen Y, Whale T, Babiuk LA, Mehtali M, et al. Replication-defective bovine adenovirus type 3 as an expression vector. *J Virol* 1999;**73**:9137–44.

116. Du E, Tikoo SK. Efficient replication and generation of recombinant bovine adenovirus-3 in nonbovine cotton rat lung cells expressing I-SceI endonuclease. *J Gene Med* 2010;**12**:840–7.
117. van Olphen AL, Mittal SK. Development and characterization of bovine x human hybrid cell lines that efficiently support the replication of both wild-type bovine and human adenoviruses and those with E1 deleted. *J Virol* 2002;**76**:5882–92.
118. van Olphen AL, Tikoo SK, Mittal SK. Characterization of bovine adenovirus type 3 E1 proteins and isolation of E1-expressing cell lines. *Virology* 2002;**295**:108–18.
119. Bangari DS, Sharma A, Mittal SK. Bovine adenovirus type 3 internalization is independent of primary receptors of human adenovirus type 5 and porcine adenovirus type 3. *Biochem Biophys Res Commun* 2005;**331**:1478–84.
120. Li X, Bangari DS, Sharma A, Mittal SK. Bovine adenovirus serotype 3 utilizes sialic acid as a cellular receptor for virus entry. *Virology* 2009;**392**:162–8.
121. Sharma A, Tandon M, Ahi YS, Bangari DS, Vemulapalli R, Mittal SK. Evaluation of cross-reactive cell-mediated immune responses among human, bovine and porcine adenoviruses. *Gene Ther* 2010;**17**:634–42.
122. Sharma A, Bangari DS, Tandon M, Pandey A, HogenEsch H, Mittal SK. Comparative analysis of vector biodistribution, persistence and gene expression following intravenous delivery of bovine, porcine and human adenoviral vectors in a mouse model. *Virology* 2009;**386**:44–54.
123. Sharma A, Bangari DS, Tandon M, HogenEsch H, Mittal SK. Evaluation of innate immunity and vector toxicity following inoculation of bovine, porcine or human adenoviral vectors in a mouse model. *Virus Res* 2010;**153**:134–42.
124. Tandon M, Sharma A, Vemula SV, Bangari DS, Mittal SK. Sequential administration of bovine and human adenovirus vectors to overcome vector immunity in an immunocompetent mouse model of breast cancer. *Virus Res* 2012;**163**:202–11.
125. Sharma A, Bangari DS, Vemula SV, Mittal SK. Persistence and the state of bovine and porcine adenoviral vector genomes in human and nonhuman cell lines. *Virus Res* 2011;**161**:181–7.
126. Zakhartchouk AN, Reddy PS, Baxi M, Baca-Estrada ME, Mehtali M, Babiuk LA, et al. Construction and characterization of E3-deleted bovine adenovirus type 3 expressing full-length and truncated form of bovine herpesvirus type 1 glycoprotein gD. *Virology* 1998;**250**:220–9.
127. Zakhartchouk AN, Pyne C, Mutwiri GK, Papp Z, Baca-Estrada ME, Griebel P, et al. Mucosal immunization of calves with recombinant bovine adenovirus-3: induction of protective immunity to bovine herpesvirus-1. *J Gen Virol* 1999;**80**(Pt 5):1263–9.
128. Baxi MK, Deregt D, Robertson J, Babiuk LA, Schlapp T, Tikoo SK. Recombinant bovine adenovirus type 3 expressing bovine viral diarrhea virus glycoprotein E2 induces an immune response in cotton rats. *Virology* 2000;**278**:234–43.
129. Kumar P, Ayalew LE, Godson DL, Gaba A, Babiuk LA, Tikoo SK. Mucosal immunization of calves with recombinant bovine adenovirus-3 coexpressing truncated form of bovine herpesvirus-1 gD and bovine IL-6. *Vaccine* 2014;**32**:3300–6.
130. Brownlie R, Kumar P, Babiuk LA, Tikoo SK. Recombinant bovine adenovirus-3 co-expressing bovine respiratory syncytial virus glycoprotein G and truncated glycoprotein gD of bovine herpesvirus-1 induce immune responses in cotton rats. *Mol Biotechnol* 2015;**57**:58–64.
131. 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.

132. Bangari DS, Mittal SK. Porcine adenovirus serotype 3 internalization is independent of CAR and alpha(v)beta(3) or alpha(v)beta(5) integrin. *Virology* 2005;**332**:157–66.
133. Bangari DS, Mittal SK. Porcine adenoviral vectors evade preexisting humoral immunity to adenoviruses and efficiently infect both human and murine cells in culture. *Virus Res* 2004;**105**:127–36.
134. Zakhartchouk A, Zhou Y, Tikoo SK. A recombinant E1-deleted porcine adenovirus-3 as an expression vector. *Virology* 2003;**313**:377–86.
135. Hammond JM, Johnson MA. Porcine adenovirus as a delivery system for swine vaccines and immunotherapeutics. *Vet J* 2005;**169**:17–27.
136. Hammond JM, McCoy RJ, Jansen ES, Morrissy CJ, Hodgson AL, Johnson MA. Vaccination with a single dose of a recombinant porcine adenovirus expressing the classical swine fever virus gp55 (E2) gene protects pigs against classical swine fever. *Vaccine* 2000;**18**:1040–50.
137. Hammond JM, Jansen ES, Morrissy CJ, Goff WV, Meehan GC, Williamson MM, et al. A prime-boost vaccination strategy using naked DNA followed by recombinant porcine adenovirus protects pigs from classical swine fever. *Vet Microbiol* 2001;**80**:101–19.
138. Hammond JM, Jansen ES, Morrissy CJ, van der HB, Goff WV, Williamson MM, et al. Vaccination of pigs with a recombinant porcine adenovirus expressing the gD gene from pseudorabies virus. *Vaccine* 2001;**19**:3752–8.
139. 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.
140. Nagy M, Tuboly T. Porcine adenoviruses: an update on genome analysis and vector development. *Acta Vet Hung* 2000;**48**:491–9.
141. Tuboly T, Nagy E. Construction and characterization of recombinant porcine adenovirus serotype 5 expressing the transmissible gastroenteritis virus spike gene. *J Gen Virol* 2001;**82**:183–90.
142. Loser P, Hofmann C, Both GW, Uckert W, Hillgenberg M. Construction, rescue, and characterization of vectors derived from ovine atadenovirus. *J Virol* 2003;**77**:11941–51.
143. Kumin D, Hofmann C, Rudolph M, Both GW, Loser P. Biology of ovine adenovirus infection of nonpermissive cells. *J Virol* 2002;**76**:10882–93.
144. Loser P, Kumin D, Hillgenberg M, Both GW, Hofmann C. Preparation of ovine adenovirus vectors. *Methods Mol Med* 2002;**69**:415–26.
145. Xu ZZ, Hyatt A, Boyle DB, Both GW. Construction of ovine adenovirus recombinants by gene insertion or deletion of related terminal region sequences. *Virology* 1997;**230**:62–71.
146. Wuest T, Both GW, Prince AM, Hofmann C, Loser P. Recombinant ovine atadenovirus induces a strong and sustained T cell response against the hepatitis C virus NS3 antigen in mice. *Vaccine* 2004;**22**:2717–21.
147. Rothel JS, Boyle DB, Both GW, Pye AD, Waterkeyn JG, Wood PR, et al. Sequential nucleic acid and recombinant adenovirus vaccination induces host-protective immune responses against *Taenia ovis* infection in sheep. *Parasite Immunol* 1997;**19**:221–7.
148. Bridgeman A, Roshorm Y, Lockett LJ, Xu ZZ, Hopkins R, Shaw J, et al. Ovine atadenovirus, a novel and highly immunogenic vector in prime-boost studies of a candidate HIV-1 vaccine. *Vaccine* 2009;**28**:474–83.
149. Rosario M, Hopkins R, Fulkerson J, Borthwick N, Quigley MF, Joseph J, et al. Novel recombinant *Mycobacterium bovis* BCG, ovine atadenovirus, and modified vaccinia virus Ankara vaccines combine to induce robust human immunodeficiency virus-specific CD4 and CD8 T-cell responses in rhesus macaques. *J Virol* 2010;**84**:5898–908.

150. Loser P, Hillgenberg M, Arnold W, Both GW, Hofmann C. Ovine adenovirus vectors mediate efficient gene transfer to skeletal muscle. *Gene Ther* 2000;**7**:1491–8.
151. Martiniello-Wilks R, Dane A, Mortensen E, Jeyakumar G, Wang XY, Russell PJ. Application of the transgenic adenocarcinoma mouse prostate (TRAMP) model for pre-clinical therapeutic studies. *Anticancer Res* 2003;**23**:2633–42.
152. Tang R, Li K, Wilson M, Both GW, Taylor JA, Young SL. Potent antietumor immunity in mice induced by vaccination with an ovine atadenovirus vector. *J Immunother* 2012;**35**:32–41.
153. Hofmann C, Loser P, Cichon G, Arnold W, Both GW, Strauss M. Ovine adenovirus vectors overcome preexisting humoral immunity against human adenoviruses in vivo. *J Virol* 1999;**73**:6930–6.
154. Morrison MD, Onions DE, Nicolson L. Complete DNA sequence of canine adenovirus type 1. *J Gen Virol* 1997;**78**(Pt 4):873–8.
155. Spibey N, McClory RS, Cavanagh HM. Identification and nucleotide sequence of the early region 1 from canine adenovirus types 1 and 2. *Virus Res* 1989;**14**:241–55.
156. Morrison MD, Reid D, Onions D, Spibey N, Nicolson L. Generation of E3-deleted canine adenoviruses expressing canine parvovirus capsid by homologous recombination in bacteria. *Virology* 2002;**293**:26–30.
157. Klonjkowski B, Gilardi-Hebenstreit P, Hadchouel J, Randrianarison V, Boutin S, Yeh P, et al. A recombinant E1-deleted canine adenoviral vector capable of transduction and expression of a transgene in human-derived cells and in vivo. *Hum Gene Ther* 1997;**8**:2103–15.
158. Kremer EJ, Boutin S, Chillon M, Danos O. Canine adenovirus vectors: an alternative for adenovirus-mediated gene transfer. *J Virol* 2000;**74**:505–12.
159. Chartier C, Degryse E, Gantzer M, Dieterle A, Pavirani A, Mehtali M. Efficient generation of recombinant adenovirus vectors by homologous recombination in *Escherichia coli*. *J Virol* 1996;**70**:4805–10.
160. Ibanes S, Kremer EJ. Canine adenovirus type 2 vector generation via I-Sce1-mediated intracellular genome release. *PLoS One* 2013;**8**:e71032.
161. Hemminki A, Kanerva A, Kremer EJ, Bauerschmitz GJ, Smith BF, Liu B, et al. A canine conditionally replicating adenovirus for evaluating oncolytic virotherapy in a syngeneic animal model. *Mol Ther* 2003;**7**:163–73.
162. Le LP, Rivera AA, Glasgow JN, Ternovoi VV, Wu H, Wang M, et al. Infectivity enhancement for adenoviral transduction of canine osteosarcoma cells. *Gene Ther* 2006;**13**:389–99.
163. Bru T, Salinas S, Kremer EJ. An update on canine adenovirus type 2 and its vectors. *Viruses-Basel* 2010;**2**:2134–53.
164. Soudais C, Boutin S, Hong SS, Chillon M, Danos O, Bergelson JM, et al. Canine adenovirus type 2 attachment and internalization: coxsackievirus-adenovirus receptor, alternative receptors, and an RGD-independent pathway. *J Virol* 2000;**74**:10639–49.
165. Chillon M, Kremer EJ. Trafficking and propagation of canine adenovirus vectors lacking a known integrin-interacting motif. *Hum Gene Ther* 2001;**12**:1815–23.
166. Zussy C, Salinas S. Study of adenovirus and CAR axonal transport in primary neurons. *Methods Mol Biol* 2014;**1089**:71–8.
167. Soudais C, Laplace-Builhe C, Kissa K, Kremer EJ. Preferential transduction of neurons by canine adenovirus vectors and their efficient retrograde transport in vivo. *FASEB J* 2001;**15**:2283–5.
168. Soudais C, Skander N, Kremer EJ. Long-term in vivo transduction of neurons throughout the rat CNS using novel helper-dependent CAV-2 vectors. *FASEB J* 2004;**18**:391–3.

169. Morante-Oria J, Carleton A, Ortino B, Kremer EJ, Fairen A, Lledo PM. Subpallial origin of a population of projecting pioneer neurons during corticogenesis. *Proc Natl Acad Sci USA* 2003;**100**:12468–73.
170. Keriel A, Rene C, Galer C, Zabner J, Kremer EJ. Canine adenovirus vectors for lung-directed gene transfer: efficacy, immune response, and duration of transgene expression using helper-dependent vectors. *J Virol* 2006;**80**:1487–96.
171. Mitani K, Graham FL, Caskey CT, Kochanek S. Rescue, propagation, and partial purification of a helper virus-dependent adenovirus vector. *Proc Natl Acad Sci USA* 1995;**92**:3854–8.
172. Serratrice N, Cubizolle A, Ibanes S, Mestre-Frances N, Bayo-Puxan N, Creyssels S, et al. Corrective GUSB transfer to the canine mucopolysaccharidosis VII cornea using a helper-dependent canine adenovirus vector. *J Control Release* 2014;**181**:22–31.
173. Cubizolle A, Serratrice N, Skander N, Colle MA, Ibanes S, Gennetier A, et al. Corrective GUSB transfer to the canine mucopolysaccharidosis VII brain. *Mol Ther* 2014;**22**:762–73.
174. Ariza L, Gimenez-Llort L, Cubizolle A, Pages G, Garcia-Lareu B, Serratrice N, et al. Central nervous system delivery of helper-dependent canine adenovirus corrects neuropathology and behavior in mucopolysaccharidosis type VII mice. *Hum Gene Ther* 2014;**25**:199–211.
175. Hartley JW, Rowe WP. A new mouse virus apparently related to the adenovirus group. *Virology* 1960;**11**:645–7.
176. Lenaerts L, Verbeken E, De CE, Naesens L. Mouse adenovirus type 1 infection in SCID mice: an experimental model for antiviral therapy of systemic adenovirus infections. *Antimicrob Agents Chemother* 2005;**49**:4689–99.
177. Wigand R. Age and susceptibility of Swiss mice for mouse adenovirus, strain FL. *Arch Virol* 1980;**64**:349–57.
178. van der Veen J, Mes A. Experimental infection with mouse adenovirus in adult mice. *Arch Gesamte Virusforsch* 1973;**42**:235–41.
179. Smith K, Brown CC, Spindler KR. The role of mouse adenovirus type 1 early region 1A in acute and persistent infections in mice. *J Virol* 1998;**72**:5699–706.
180. Kajon AE, Brown CC, Spindler KR. Distribution of mouse adenovirus type 1 in intraperitoneally and intranasally infected adult outbred mice. *J Virol* 1998;**72**:1219–23.
181. Cauthen AN, Brown CC, Spindler KR. In vitro and in vivo characterization of a mouse adenovirus type 1 early region 3 null mutant. *J Virol* 1999;**73**:8640–6.
182. Meissner JD, Hirsch GN, LaRue EA, Fulcher RA, Spindler KR. Completion of the DNA sequence of mouse adenovirus type 1: sequence of E2B, L1, and L2 (18-51 map units). *Virus Res* 1997;**51**:53–64.
183. Beard CW, Spindler KR. Characterization of an 11K protein produced by early region 3 of mouse adenovirus type 1. *Virology* 1995;**208**:457–66.
184. Ying B, Smith K, Spindler KR. Mouse adenovirus type 1 early region 1A is dispensable for growth in cultured fibroblasts. *J Virol* 1998;**72**:6325–31.
185. Beard CW, Spindler KR. Analysis of early region 3 mutants of mouse adenovirus type 1. *J Virol* 1996;**70**:5867–74.
186. Raman S, Hsu TH, Ashley SL, Spindler KR. Usage of integrin and heparan sulfate as receptors for mouse adenovirus type 1. *J Virol* 2009;**83**:2831–8.
187. Lenaerts L, van DW, Persoons L, Naesens L. Interaction between mouse adenovirus type 1 and cell surface heparan sulfate proteoglycans. *PLoS One* 2012;**7**:e31454.
188. Lenaerts L, McVey JH, Baker AH, Denby L, Nicklin S, Verbeken E, et al. Mouse adenovirus type 1 and human adenovirus type 5 differ in endothelial cell tropism and liver targeting. *J Gene Med* 2009;**11**:119–27.

189. Robinson M, Li B, Ge Y, Ko D, Yendluri S, Harding T, et al. Novel immunocompetent murine tumor model for evaluation of conditionally replication-competent (oncolytic) murine adenoviral vectors. *J Virol* 2009;**83**:3450–62.
190. McCarthy MK, Procario MC, Twisselmann N, Wilkinson JE, Archambeau AJ, Michele DE, et al. Proinflammatory effects of interferon gamma in mouse adenovirus 1 myocarditis. *J Virol* 2015;**89**:468–79.
191. McCarthy MK, Levine RE, Procario MC, McDonnell PJ, Zhu L, Mancuso P, et al. Prostaglandin E2 induction during mouse adenovirus type 1 respiratory infection regulates inflammatory mediator generation but does not affect viral pathogenesis. *PLoS One* 2013;**8**:e77628.
192. Weinberg JB, Stempfle GS, Wilkinson JE, Younger JG, Spindler KR. Acute respiratory infection with mouse adenovirus type 1. *Virology* 2005;**340**:245–54.
193. Ojkic D, Krell PJ, Tuboly T, Nagy E. Characterization of fowl adenoviruses isolated in Ontario and Quebec, Canada. *Can J Vet Res* 2008;**72**:236–41.
194. Ojkic D, Nagy E. The complete nucleotide sequence of fowl adenovirus type 8. *J Gen Virol* 2000;**81**:1833–7.
195. Marek A, Nolte V, Schachner A, Berger E, Schlotterer C, Hess M. Two fiber genes of nearly equal lengths are a common and distinctive feature of Fowl adenovirus C members. *Vet Microbiol* 2012;**156**:411–7.
196. Corredor JC, Garceac A, Krell PJ, Nagy E. Sequence comparison of the right end of fowl adenovirus genomes. *Virus Genes* 2008;**36**:331–44.
197. Sheppard M, Werner W, Tsatas E, McCoy R, Prowse S, Johnson M. Fowl adenovirus recombinant expressing VP2 of infectious bursal disease virus induces protective immunity against bursal disease. *Arch Virol* 1998;**143**:915–30.
198. Renaut L, Colin M, Leite JP, Benko M, D'Halluin JC. Abolition of hCAR-dependent cell tropism using fiber knobs of Atadenovirus serotypes. *Virology* 2004;**321**:189–204.
199. Johnson MA, Pooley C, Ignjatovic J, Tyack SG. A recombinant fowl adenovirus expressing the S1 gene of infectious bronchitis virus protects against challenge with infectious bronchitis virus. *Vaccine* 2003;**21**:2730–6.
200. Johnson MA, Pooley C, Lowenthal JW. Delivery of avian cytokines by adenovirus vectors. *Dev Comp Immunol* 2000;**24**:343–54.
201. Reddy PS, Idamakanti N, Song JY, Lee JB, Hyun BH, Park JH, et al. Nucleotide sequence and transcription map of porcine adenovirus type 3. *Virology* 1998;**251**:414–26.