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Effect of administration route on biodistribution and shedding of replication-deficient viral vectors used in gene therapy

A literature study

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Abstract

Effect of administration route on biodistribution and shedding of replication-deficient viral vectors used in gene therapy
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In gene therapy, genes (heredity material) are introduced in patients to treat diseases caused by deletions or alterations in genes. With adapted viruses it is possible to direct the gene of interest to a desired place in the body. This modified virus is called a viral vector.

The gene of interest can also spread, via the viral vector, to a site outside the patient. The gene can then possibly be transferred to other people, animals and other organisms where it may cause undesired effects. To evaluate the risks of viral spreading, knowledge of how the viral vector behaves inside the body and subsequently leaves it, for example via faeces, urine or saliva, is crucial.

In this report, the distribution in and the excretion from the body of two viral vectors (HAdV-5 and AAV2) per administration route (i.e. via blood or muscles) have been critically evaluated. It can be concluded that as well as the type of viral vector involved, the administration route also influences the distribution and excretion of the viral vector. The viral vector remains local through some routes and is then only excreted via only one or a few routes. Other routes result in distribution throughout the entire body and excretion via several routes. These processes are described in qualitative models. The information from these models is relevant for researchers, risk assessors and regulators in the development of viral vectors for gene therapy.

Key words:
viral vectors, gene therapy, shedding, biodistribution, HAdV-5, AAV2

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Rapport in het kort

Effect van de toedieningsroute op de biodistributie en excretie van replicatie-deficiënte virale vectoren welke gebruikt worden bij gentherapie
Een literatuur studie

Bij gentherapie worden genen (erfelijk materiaal) bij patiënten ingebracht om ziektes te behandelen waarvan de oorzaak een ontbrekend of veranderd gen is. Met aangepaste virussen is het mogelijk om het te introduceren gen naar de gewenste plek in het lichaam te brengen. Dit veranderde virus wordt een virale vector genoemd.

Het te introduceren gen kan zich via de virale vectoren ook buiten de patiënt verspreiden. Dat kan zowel in mensen, als in dieren en andere organismen, waar het eventueel ongewenste effecten kan veroorzaken. Om de risico's van verspreiding te beoordelen is kennis nodig over de manier waarop het virus zich binnen het lichaam gedraagt en over hoe het vervolgens het lichaam verlaat, via bijvoorbeeld ontlasting, urine of speeksel.

In dit rapport wordt van twee virale vectoren (HAdV-5 en AAV2) per toedieningsroute, via bijvoorbeeld het bloed of de spieren, kritisch geëvalueerd hoe zij zich in het lichaam verspreiden en hoe zij zich uitscheiden. Het blijkt dat behalve het type virale vector, de toedieningsroute invloed heeft op de verdeling en uitscheiding van de vector. Via sommige toedieningsroutes blijft de virale vector lokaal en wordt hij via één of enkele routes uitgescheiden. Andere toedieningsroutes resulteren in verspreiding over het hele lichaam en uitscheiding via meerdere routes. Deze processen zijn beschreven in kwalitatieve modellen. De informatie uit deze modellen is relevant voor onderzoekers, risicobeoordelaars en het beleid bij de ontwikkeling van virale vectoren voor gentherapie.

Trefwoorden:
virale vectoren, gentherapie, uitscheiding, biodistributie, HAdV-5, AAV2

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Abbreviations

Ad	adenovirus
HAdV-5	human adenovirus serotype 5
AAV	adeno-associated virus
AAV2	adeno-associated virus serotype 2
ADME	absorption, distribution, metabolism and excretion
BAL	bronchoalveolar lavage
CAR	coxsackie/adenovirus receptor
c-Met	hepatocyte growth factor receptor
CPE	cytopathic effect assay
DNA	deoxyribonucleic acid
drp	deoxyribonuclease-resistant particles
GI	gastrointestinal tract
iu	infectious unit
kg	kilogram
LGN	lateral geniculate nucleus
PBK	physiology based kinetic
PCR	polymerase chain reaction
pfu	plaque forming unit
Q-PCR	quantitative polymerase chain reaction
RGD	integrin binding arginine–glycine–aspartate
RT-PCR	reverse transcriptase polymerase chain reaction
ru	replication units
vp	vector particle

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Summary

Gene therapy is a rapidly developing field in which genes are introduced to treat diseases caused by deletions or alterations in DNA sections (*e.g.* haemophilia). Viral vectors are a frequently used form of gene therapy to introduce therapeutic genes. A wide range of viral vectors is used in the field of gene therapy, for example HAdV-5 (an adenovirus) and AAV2 (an adeno associated virus). Each viral vector has its specific safety profile with respect to the risks of infection with the viral vector for the patient and his environment, including people in close contact with the patient. To evaluate this risk, knowledge on the biodistribution and potential shedding (= excretion) of the viral vector is crucial.

The current public literature was analysed for biodistribution and shedding data for adenovirus and adeno-associated virus from preclinical and clinical studies with a focus on the influence of the administration route on spreading. Studies describing *ex vivo* gene therapy are not included and their biodistribution and risk of shedding are beyond the scope of this report.This report will only focus on HAdV-5 and AAV2, the serotypes mostly used in viral gene therapy. Based on biodistribution and shedding data related to the used administration route, descriptive qualitative models were formulated for the biodistribution and shedding of viral vectors.

This report shows that biodistribution and shedding via excreta depend on the route of administration. Some routes lead to local biodistribution (*e.g.* intraperitoneal) and thus to no shedding or local shedding only. Other routes lead to systemic biodistribution (*e.g.* intra-muscular) and to shedding via several excretion routes (*e.g.* urine and faeces). The most striking differences between both vectors were observed for shedding via semen en the transport over the blood-brain barrier. Shedding via semen can be expected for AAV2, but not for HAdV-5. In addition, transport across the blood-brain barrier is expected for AAV2 but not for HAdV-5, given that the blood-brain barrier is intact.

Based on the obtained biodistribution and shedding data, it is not possible to formulate a quantitative model at this moment, because it is not possible to generate kinetic input parameters of HAdV-5 or AAV2 for such a model. For a quantitative modelling approach, there is need for more experimental data on biodistribution and shedding, e.g. from sampling blood, tissues (if possible) and excreta at multiple time points from shortly after administration to a period of several weeks or months. However, it was possible to construct qualitative models for HAdV-5 and AAV2 and describe the biodistribution and shedding after administration via various routes. These models can help researchers and risk assessors in predicting the different shedding routes after a certain administration route. In addition, the qualitative models can help researchers to setup relevant pre-clinical *in vivo* experiments and clinical trials. In addition, they can help risk assessors to determine the risk of shedding via the different excretion routes. Finally, it can help regulators in setting up guidance for non-clinical studies and clinical trials.

Biodistribution of gene therapy viral vectors is mostly investigated in animal models. Differences between species in receptors needed for viral infection, erythrocyte binding or the presence of neutralising antibodies can influence the biodistribution profile and the shedding. It is important to be aware of these differences when the spreading of an adenoviral vector from an animal model is extrapolated to humans. However, good (pre-)clinical kinetic studies are needed, in which biodistribution and shedding is investigated in the relevant tissues and excreta routes and for a relevant time frame.

1 Introduction

1.1 Gene therapy

Gene therapy is a rapidly developing field in which genes are introduced to individuals to treat diseases caused by deletions or alterations in DNA (*e.g.* haemophilia, cystic fibrosis, cancer). Viral vectors are a frequently used form of gene therapy to introduce therapeutic genes in the tissue or at cellular level. Although gene therapy testing in humans has advanced rapidly, many questions surround its use.
A wide range of viral vectors is used in the field of gene therapy. Among those are vectors derived from adenovirus, retrovirus, adeno-associated virus (AAV) and poxvirus like vaccinia virus and canary pox virus (http://www.wiley.co.uk/genmed/clinical). Each viral vector shows a specific safety profile with respect to the risks that might occur when a vector is administered to an animal or a patient. Essential vector properties such as the molecular design, the expression cassette and expression of the cloned transgene will affect this safety profile. In order to generate a safety profile for a particular use of a certain vector, it is essential to evaluate the risks based on all essential elements, including the administration route. In addition, not only the risk for the patient needs to be assessed, but also the risk for the workers involved and other people in close contact needs to be evaluated. To evaluate this risk, knowledge on the potential spreading of the viral vector is crucial.
A recently published definition of biodistribution and shedding is applied in this report (1). Biodistribution is defined as the spreading of a viral vector in tissues and organs of the treated individual (animal or human) after administration of the viral vector. Shedding is defined as the dissemination of a vector outside the patient and thus into the environment. Shedding routes include urine, faeces, sweat, saliva, nasopharyngeal fluids, breath, semen, menstrual blood and wound exudates. Plasma and blood are not excreta, because under normal conditions it is not shed spontaneously. Except for shedding via wound exudates and menstrual blood, it is expected that shedding into the environment can only occur after the vector is delivered to organs that play a role in excretion. Therefore, biodistribution data are not only useful to evaluate patient safety, but may also predict shedding of a viral vector into the environment.
To evaluate this potential spreading, information on biodistribution and shedding should be assessed for each viral vector. Spreading of viral vectors is influenced by several factors like: 1) the quantity of the vector delivered to the subject and 2) the susceptibility of different tissues to viral infection. To evaluate the safety profile of viral derived vectors, frequently used in gene therapy, knowledge on wild type virus should be included.

1.2 Goal

Regarding safety and risk assessment of viral vectors used in gene therapy, biodistribution and shedding play a pivotal role. In this report, biodistribution and shedding data from pre-clinical and clinical studies obtained with two non-replicating viral vectors (HAdV-5 and AAV2, was analysed. These viral vectors were selected, because they are the most frequently used non-replicating viral vector serotypes. Current public literature was analysed with a focus on the influence of the administration route on spreading. Studies describing *ex vivo* gene therapy are not included and their biodistribution and risk of shedding are beyond the scope of this report.

This report presents a critical overview on biodistribution and shedding data related to the used administration route. Based on these data, it will be attempted to formulate a model for the biodistribution and shedding of viral vectors. These models can help researchers and risk assessors in predicting the different shedding routes after a certain administration route. However, a modelling approach for the biokinetics of viral vectors, which are by no means 'simple' chemical compounds, is more difficult because of the more complex behaviour of the viral vectors relative to the well understood physicochemical processes of chemical compounds. Inaddition, there is a great number of different viruses and serotypes used in gene therapy. Therefore, 'a' model for viral vectors, considered together as a 'compound' (be it biological of nature rather than chemical) is not realistic, but a model per viral vector serotype should be constructed. If a quantitative model per viral vector serotype is not feasible then a descriptive qualitative model will be formulated.

1.3 Adenovirus (Ad)

1.3.1 Wild type virus

There are 51 serotypes of human adenoviruses, classified into 6 species (A-F) (2;3). Adenovirus group C infections occur worldwide in humans and HAdV-5 infections are usually acquired during childhood. Subgroup B serotype infections in the human population are rare, resulting in a much lower seroprevalence (4). Although epidemiologic characteristics of the adenoviruses vary by type, all are transmitted by direct contact, faecal-oral transmission, and occasionally by waterborne transmission. Adenoviruses mostly enter their hosts by mouth, the nasopharynx or the ocular conjunctiva. Most adenovirus infections are locally in the eyes, the pharynx or lungs (5). Adenoviruses can be present for a longer period in intestine and faeces are considered a common source of shedding. Latency of adenoviruses was observed in tonsils and lymphocytes, because re-isolation of latent adenovirus could be demonstrated 24 months after initial infection. Persistence of adenoviruses in lymphocytes of a human host can last for years (6). Two viral gene products (E1A and E3) facilitate persistence by antagonising antiviral responses of the host (7). Replication of adenoviruses preferentially occurs in cells of the respiratory epithelium, although limited replication in lymphocytes, the gastrointestinal tract, urinary bladder and liver has been reported. Additionally, culturing of adenoviruses from the blood suggests a viremic spread (8).

Adenoviruses are species specific; however, some crosses of the species barrier have been reported. For HAdV-5, infection of experimental animals (cotton rats) can occur through lungs (9) and eyes (10). Upon infection in these animals, also shedding of adenovirus was observed. In most other species, like mice, adenoviruses fail to produce new virus particles, although the animals could be infected and showed expression of viral proteins (5).

1.3.2 Attachment and entry of adenoviral vectors

Attachment and entry of wild type adenovirus and adenoviral vectors requires binding of viral proteins to cellular receptors. Attachment of most human adenoviruses (including HAdV-5) involves high affinity binding of the viral capsid protein to the coxsackie/adenovirus receptor (CAR) (11-14). Cell entry through internalisation in coated pits is then mediated by the binding of adenovirus to cell surface RGD-binding integrins (14-16). The role of CAR and RGD binding integrins is supported by the observation that various cell lines which lack CAR or RGD binding integrins are not effectively infected with adenoviral vectors (11;16;17). Also *in vivo* spreading routes are influenced by the presence or absence of CAR (13). To extrapolate the spreading of an adenoviral vector from an animal model to humans, it is important to be aware of the differences in CAR distribution. CAR expression is widespread in both human and mice, being present in many organs including heart, brain, pancreas, liver, lung, kidney, small intestine, colon and prostate (12;13;17-19). However, it has been reported that while CAR is present on human erythrocytes, it is not on mice erythrocytes (20). This would explain a

high adenoviral vector concentration in mouse plasma while, as a result of vector binding to human erythrocytes, human plasma contains a significantly lower adenoviral vector concentration. Therefore, it should be noted that in order to determine the actual amount of adenoviral vectors in human blood, analysis of erythrocyte samples is required in addition to only plasma material.
Unlike HAdV species A, C, D E and F, species B and some species D do not bind to CAR, but use the membrane cofactor CD46 as attachment receptor (4;21). CD46 is ubiquitously expressed in almost all human cells and although there is a high conservation between human CD46 and CD46 of monkeys (both old and new world), conservation between human and pigs, guinea pigs, rats and mice is very low (4).
The differences in humans and animals with regard to receptor expression and conservation emphasize that one should be careful when results obtained with HAdVs in animal studies are extrapolated to the human situation.

1.4 Adeno-associated virus (AAV)

1.4.1 Wild type virus

AAV belongs to the Parvoviridae family which belongs to the smallest animal DNA viruses. Because of the frequent association with adenoviruses these viruses were named adeno-associated virus (22). Different AAV serotypes have been isolated from several avian and mammalian hosts. Human infections with AAV are very common and AAV serotype 2 is prevalent in humans. It has been reported that 85-90% of the human population is sero-positive for antibodies against AAV (23;24). Tissue specificity is determined by the capsid serotype. AAV2 presents natural tropism towards skeletal muscles, neurons, vascular smooth muscle cells and hepatocytes (25)
AAV2 is detected in blood, genital tract, cervical epithelium and semen (26-28). Additionally Burguete *et al*. presented evidence for infection of the human embryo with AAV during pregnancy (29). The evidence was based on the presence of AAV virions in amnion fluid. In mice, transplacental transmission of AAV1 has been reported (30;31).
Replication of AAV is dependent on the cellular function and requires the cell to go through S phase. Furthermore, the replication of AAV is dependent on co-infection with a helper virus such as adenovirus or herpes virus. In the absence of a helper virus co-infection the AAV genome can integrate into the cellular DNA and as such establish a latent infection. Latent AAV infection can be reactivated upon infection with a helper virus.

1.4.2 Attachment and entry of AAV vectors

Heparan sulphate proteoglycans serve as primary attachment receptor for AAV2 (32). The presence of heparan sulphate proteoglycans on the cell surface directly correlates with the efficiency by which AAV can infect cells. The use of heparan sulphate proteoglycans as receptor explains the broad tropism of AAV, which include human, non-human primate, canine, murine and avian cell types. It should be noted that removal of heparan sulphate moieties from the cell surface did not completely abolish AAV infectivity. AAV still exhibits some specific binding to cell lines that do not produce heparan sulphate proteoglycans, indicating that in the absence of this receptor AAV attachment and infection can occur although inefficiently (32).
Upon receptor binding of AAV2, a co-receptor is required to enable internalisation. It has been reported that αvβ5 integrin heterodimers, hepatocyte growth factor receptor c-Met and fibroblast growth factor receptor 1 serve as suitable co-receptors (33;34). The αvβ5 integrins also act as a co-receptor for HAdV-5. c-Met is predominantly expressed in epithelial cells and in several non-epithelial cells, such as liver, neural, and skeletal muscle cells (35). Those cells are successfully transduced by AAV2 (36). Fibroblast growth factor receptor 1 is expressed in many organs and tissues, however, the relative abundance in skeletal muscle, neuroblasts and glioblasts in the brain correlates with the documented high efficiency of AAV-mediated transduction in these tissues (37;38).

1.5 Quantitative modelling

Prediction of the human *in vivo* kinetics based on *in silico*, *in vitro* and animal data can be very helpful in the field of risk assessment (species to species extrapolation, high dose to low dose extrapolation, route to route extrapolation, *in vitro-in vivo* extrapolation). In the field of gene therapy, it can help researchers in estimating the concentration of the viral vector at the site of action and possible adverse effects in the patient. In addition, it can help risk assessors in predicting the risk for the patients environment. Human *in vivo* kinetics can be predicted with quantitative models. There are several different modelling methods, for example physiologically based kinetic (PBK) modelling.
PBK modelling can predict *in vivo* kinetics by combining data from various sources (*in vivo*, *in vitro* and *in silico*). PBK models are compartment models where transfer of a compound between the body compartments and its elimination from the body system is described in terms of the physiological properties of the species of interest (*e.g.* human or laboratory animal) and physicochemical and biochemical properties of the compound. An example of such a PBK model is shown in figure 1. PBK modelling accounts at least for the gross physiological aspects of the biological system such as organ volumes, regional blood flow and alveolar flow. But these models are flexible enough to incorporate more refined structural compartimentation and biochemical processes. In addition, detailed kinetic input, such as information on the distribution rate and complexation with the immune system, is needed to make a model which can make a reliable prediction for the human *in vivo* kinetics. The different ADME (absorption, distribution, metabolism and excretion) processes are incorporated into these models. Distribution of a substance is generally assumed to be determined by tissue composition and physicochemical properties of the substance such as solubility in water and octanol (as a representation of body fat) and binding to plasma proteins. However, the distribution of substances that are not only governed by the physicochemical properties of the substance and tissue components, but also by active processes through membrane transporters, can be modelled. Further elimination of the parent compound is by renal, bile and respiratory clearance. Also formation of immune complexes and thus the elimination via an immune reaction can be incorporated in a quantitative model. The level of detail of a PBK model highly depends on the availability of experimental data and/or detailed knowledge of the physical-chemical properties of a compound.
To generate a PBK model for the viral vectors HAdV-5 and AAV2, kinetic input parameters for absorption, distribution and excretion are needed per administration route. For input on the absorption and distribution, quantitative information on the biodistribution versus time has to be extracted from the current public literature. It is not expected that viral vectors are metabolised such as chemical compounds. Shedding data from various relevant time points and data on immune-complex formation are needed to be incorporated in the excretion part in the PBK model.

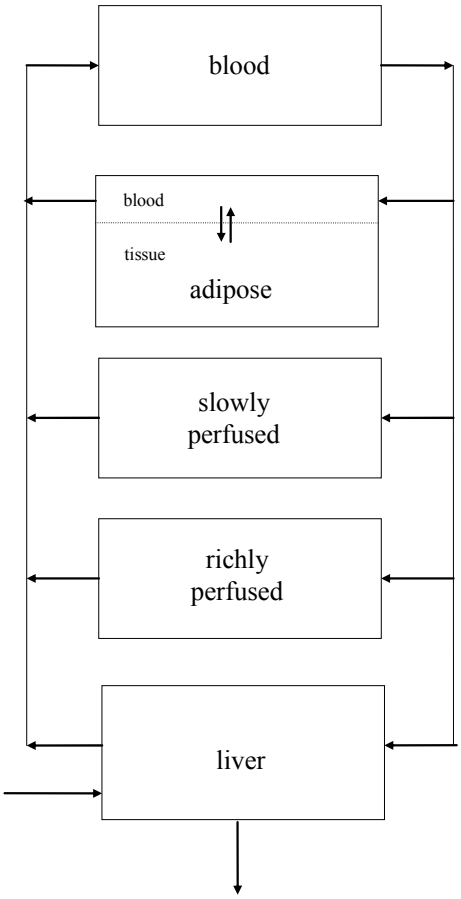

Figure 1. Example of a PBK model for oral exposure. The squares represent different tissues (compartments) relevant for the kinetics of a certain compound. The arrows represent the transport of the compound between the different tissues.

2 Method

PubMed was searched for articles using the following keywords: gene therapy, viral, viral vector, virus, vaccine, adenovirus/adenoviral/Ad and adeno-associated virus/adeno-associated viral/AAV in combination with kinetic/kinetics, shedding, excretion, biodistribution/distribution, model and modeling/modelling published until 31 December 2007. Based on the title and the abstract, a selection was made retrieving all articles that described pre-clinical or clinical trials with adenoviral or adeno-associated viral vectors and modelling of viral kinetics.
For adenoviral and adeno-associated viral vectors 151 and 89 articles were selected, respectively. The content of these articles was analysed for information on biodistribution and shedding and also for relevant references. A second selection was on replication-deficient vectors and on serotype HAdV-5 and AAV2. Thus, replication competent adenoviral vectors were excluded. Articles on other serotypes and on viral vectors designed to change the vector tropism were excluded, due to the possibility of altered biodistribution and shedding properties. The information on biodistribution and shedding was categorised based on the applied administration route.
Sixteen articles were found describing a model for viruses or vaccines, not selectively on adenovirus and adeno-associated virus. These articles were screened for relevance, quantitative biodistribution and shedding data at several time points per viral vector and administration route, for the development of a PBK model for adenoviruses and adeno-associated viruses used in gene therapy.

3 Results

3.1 Analysis

3.1.1 Viral titres

Viral titres were expressed in various ways in the reviewed studies used. Basically three types of titres can be distinguished: vector particles, DNase resistant particles and infecting units. Vector particles (vp) or genome particles are measured with PCR and only provide information concerning the amount of genome copies. This can include unpackaged DNA and DNA from defective viral particles. DNase resistant particles (drp) on the other hand only provide information regarding correctly folded viral capsids. This can again include defective viral particles as well as empty capsids. Unpackaged DNA, empty capsids and defective viral particles are not able to deliver vector DNA to (mammalian) cells and therefore overestimate the 'real' viral titre. Infectious units (iu) give the most accurate information about the amount of viral particles that are able to introduce their therapeutic genes at the tissue or cellular level. Various infectious assays can be used to determine a viral titre; the assay mostly used is the plaque forming assay, which provides a titre in plaque forming units (pfu).

3.1.2 Assays

Furthermore, biodistribution and shedding were measured using a variety of assays, ranging from PCR to *in vivo* imaging. The various assays used in the references can be found in Table 1. When data from infectious assays were available, these were used since they have the most relevance for risk assessment purposes. If infectious assay data were not available, the following data were used in order of importance: Q-PCR, non-Q-PCR, PCR not specified, RT-PCR, Southern blot, transgene expression and *in vivo* imaging. Antibody assay data were not used, because they do not provide information on the presence of viral particles in specific biodistribution or shedding samples. In addition, one has to keep in mind that in most cases transgene expression and RT-PCR only give information on the expression of the gene product and not on the localisation of the viral vector itself.

Table 1. Assays used in references to determine biodistribution and shedding

	HAdV-5 (n = 53 references)		**AAV2 (n = 31 references)**	
type of assay	**biodistribution**	**shedding**	**biodistribution**	**shedding**
PCR (total)	38 (72%)	16 (30%)	22 (71%)	9 (29%)
PCR not specified	*25 (47%)*	*14 (26%)*	*12 (39%)*	*6 (19%)*
Q-PCR	*7 (13%)*		*7 (23%)*	*2 (6%)*
non-Q-PCR				*1 (3%)*
RT-PCR	*6 (11%)*	*2 (4%)*	*3 (10%)*	
Southern blot	2 (4%)	1 (2%)	6 (19%)	
antibody assay	2 (4%)	2 (4%)	2 (6%)	
transgene expression	13 (25%)	4 (8%)	1 (3%)	
infectious assay	14 (26%)	14 (26%)	3 (10%)	3 (10%)
in vivo imaging	2 (4%)			

3.1.3 Administration route

Currently, HAdV-5 derived vectors have been administered using different administration routes, such as intravenous, intra-muscular, intraperitoneal, intra-prostatical, dermal, intra-tumoral, brain, inhalatory and intranasal-bronchial. In addition, data on some minor routes of administration were found. The following administration routes were found in literature for AAV2 derived vectors: intravenous, intraperitoneal, intra-muscular, inhalatory and intranasal-bronchial. Also data on other routes of administration were found, but for these routes only limited literature data was available.

3.2 Results HAdV-5

Biodistribution and shedding of HAdV-5 viral vectors are dependent on the route of administration. In table 2 the biodistribution and shedding routes of HAdV-5 viral vectors following various administration routes are described. Detailed information on the biodistribution and shedding per experiment can be found in Appendices A (Table A.1 to A.15) and B.

Table 2. Biodistribution and shedding of HAdV-5

Administration route	Biodistribution	Shedding
intravenous	*positive*: blood, blood cells, plasma, serum, heart, kidney, liver, lung, spleen, prostate, GI-tract, muscle, bladder, ureter, bone marrow, eye, gonads, lymph nodes and pancreas	*positive*: bile, rectal swab, urine and sperm (probably due to blood in sample)
	negative: brain	*negative*: oral rinse
intraperitoneal and intra-pleural	*positive*: ascites, pleural fluid, kidney, liver, prostate and spleen	
	negative: lung and blood	*negative*: sputum, stool, urine, nasal swab, urethral swab and rectal swab
intra-tumoral	*positive*: plasma and proximal lymph node	*positive*: urine, saliva, sputum, throat swab, faeces and bronchial fluid
	negative: distal lymph nodes, liver, kidney and testis	
dermal	*positive*: axillary lymph node	*positive*: wound bed
	negative: blood, brain, gonads, heart, kidney, liver, lung, spleen and mesenteric lymph nodes	
intra-prostatic	*positive*: blood, prostate, heart, kidney, liver, lung, spleen, GI-tract, bladder, testis, epididymis, rectum, muscle, thyroid and salivary glands	*positive*: urine
		negative: stool, saliva, ear swab and nasal swab

Table 2. Biodistribution and shedding of HAdV-5 (continued)

Administration route	Biodistribution	Shedding
brain	*positive*: brain *negative*: blood, serum and plasma	*negative*: urine, faeces, sputum, nasal swab and rectal swab
intra-muscular	*positive*: blood, liver, lung, spleen and heart	*negative*: urine, faeces, stool, semen and throat swab
inhalatory and intranasal-bronchial	*positive*: blood, lung, tonsil and nostril	*positive*: saliva, sputum, faeces, nasal brush, rectal swab, pharynx swab and bronchial fluid *negative*: urine
ocular	*positive*: blood, heart, kidney, liver, spleen, bone marrow, conjunctiva, LGN, optic nerve and retina *negative*: lung, gonads and brain	*negative*: sputum and urine
salivary gland	*positive*: blood, gonads, heart, liver, lung, spleen, mandibular lymph nodes and sublingual, parotid and submandibular glands *negative*: brain, kidney, intestine, tongue and oral mucosa	no data available
para-aortic lymph node	*positive*: serum	*negative*: urine
spine	*positive*: serum	*negative*: urine
ileum	*positive*: serum	*negative*: urine
bladder	*positive*: bladder, liver, kidney, heart, gonads, lung, adrenal gland and ureter	no data available
foetal	*positive:* heart, liver, spleen, adrenals, brainstem, digestive tract, kidney, lung, muscle, placenta, peritoneum and skin *negative:* cerebral cortex	no data available

GI-tract = gastrointestinal tract
LGN = lateral geniculate nucleus

3.2.1 Biodistribution and shedding per administration route

3.2.1.1 Intravenous administration

HAdV-5 viral vectors that are administered intravenously or intra-arterially are rapidly distributed via the bloodstream, irrespective of the dose injected. Already 10 minutes following administration in mice, viral vector DNA can be detected in the liver, lung and spleen and at several time points between 72 hours and 2 weeks after injection all major organs of the body contain vector DNA (39-42). Also in rabbits, viral DNA and/or RNA could be detected in eye, heart, kidney, liver, lung, ovary and spleen, one day after injection (43). Two weeks after intra-arterial administration, viral RNA was detected in the bone marrow, white blood cells, liver, lung, lymph nodes, muscles and testes of rabbits, but not in the brain and epididymis. Furthermore, depending on the infused artery, viral DNA was also found in the heart, kidney and spleen (44). In addition, in plasma samples of rabbits injected intravenously with recombinant HAdV-5 vectors, infectious particles were found from 10 minutes up to 48 hours after injection (45). In humans, vector DNA could be found in plasma up to 28 days, following injection of $1*10^{12}$ vp or more (46). When doses of $1*10^{11}$ vp or lower were injected, plasma samples became negative 5 days following administration (46). Organ samples on the other hand can remain positive in experimental animals for at least 49 weeks (last time point analysed) (47). Despite extensive distribution in rabbits, no HAdV-5 could be found in the brain, indicating that HAdV-5 is not transported over the blood-brain barrier (44). Only injection of a very high dose of viral vector, irrelevant for human therapy ($2*10^{12}$ vp/kg) into the femoral vein of baboons induced spreading of viral DNA to the brain (48).

Since Ad 5 is distributed to all organs that play a role in excretion (lungs, bladder, GI-tract and gonads) following intravenous administration (39-44;47;49-52), it can be expected that shedding will occur via several routes. Indeed, traces of viral vectors have been found in bile (10 minutes up to 24 hours) following administration of an HAdV-5 viral vector in mice (40). Sperm samples of rabbits have been tested positive, however; this was presumably caused by contamination with blood (44). In a human clinical study, occasional positive findings were reported in rectal swabs and urine somewhere between 1 and 28 days following repeated injections of $3*10^{10}$ – $3*10^{12}$ vector particles in the bloodstream (46). The presence of viral vector genome particles in blood, plasma or serum was found between 1 minute until a maximum of 28 days (41;43;45;46;53;54). However, shedding data for periods over 1 month are not present for the intravenous administration route.

3.2.1.2 Intraperitoneal and intra-pleural administration

Only limited data are available concerning the biodistribution of HAdV-5 viral vectors following intraperitoneal administration. In mice, viral DNA was found in the liver, kidney, spleen, prostate and peritoneal and pleural fluid 2 weeks after viral delivery, but not in the lung (39). In human subjects, no evidence was found for shedding via sputum, urine or faeces within 28 days after administration (55;56). Also following intra-pleural delivery of HAdV-5 vector particles, blood samples and nasal, urethral and rectal swabs remained negative for 28 days (57).

3.2.1.3 Intra-tumoral administration

It can be expected that biodistribution and shedding patterns depend on the origin of the injected tumour. In tumour models in mice, viral particles were already detected in plasma 25 seconds after injection in the tumour. Plasma values peaked during infusion and 10 minutes after the infusion few viral vectors remained in the blood. Viral DNA was also detected in the liver 10 minutes after infusion (58). Clinical trials in lung cancer patients reported the presence of viral DNA in blood or plasma samples from 30 minutes up to 7 days following injection (59-62). Later time points were not analysed. In two patients that died 25 and 151 days after the start of the gene therapy, viral DNA could not be detected in kidneys, liver, testis and distal lymph nodes. However, in 1 patient viral DNA was detected in the proximal lymph nodes (61). Shedding was consistently detected in sputum up to 60 days (59;60;62) and gargle up to 15 days (61). In some of the studies, shedding in urine was reported even

up to 14 days (59;61), however, this was not confirmed by a third study (60). After two days, shedding was detected in faeces and throat swabs of patients injected with a high dose, but not with a low dose of HAdV-5 (60). In addition, viral presence was detected in saliva in the first 24 hours, but not 9 days after viral delivery (59).
Following administration of HAdV-5 viral particles into other tumours in the interior of the body (breast, colon, liver, adrenals) virus was detected in blood or plasma between 8 and 24 hours after injection (63-65). Shedding analysis in stool, throat or nasal swabs and urine were negative (63-65). Cunningham et al. (2005) showed that the injection site remains positive for 30 days after injection of $2*10^{10}$ to $2*10^{12}$ vp in humans with carcinomas (66). Blood, sputum and urine samples taken at 2 weeks were negative when HAdV-5 viral vector particles were delivered into soft tissue sarcomas of the extremities (67). Repeated administration of doses larger than $3*10^{10}$ pfu in patients with squamous cell carcinomas of head and neck resulted in the appearance of infectious particles in blood 30 minutes up to 24 hours after injection of the virus into the tumour. Viral DNA was detected in a dose dependent manner. In addition, infectious HAdV-5 was detected in urine from patients who received doses of $3*10^{9}$ pfu or greater and HAdV-5 was detected in sputum and saliva of patients who received doses of $1*10^{11}$ pfu (68). After direct injection into a brain tumour, no viral genome particles could be detected in blood or serum or in the shedding routes urine and nasal swab (69;70). This confirms that HAdV-5 is not transported over the blood-brain barrier.

3.2.1.4 Dermal administration
In rabbits, where 4 weekly dosages were administered to the surface of a wound, data indicate that the viral particles did not reach the bloodstream and remained near the surface of the wound. Organs remained negative and only the wound bed and nearby lymph nodes tested positive 22 days after the first injection (71). Data with regard to shedding following dermal administration are not available, but no viral DNA in blood and organs was detected.

3.2.1.5 Intra-prostatic administration
In humans, after vector doses up to $1*10^{11}$ iu, no viral DNA could be detected in blood, plasma or serum (54;72;73). No human biodistribution data are available however. Data in mice showed biodistribution of intra-prostatic administered HAdV-5 to the liver and prostate, but not to lung, kidney or spleen 14 days after injection, whereas in studies with dogs, spreading to a variety of organs including prostate, kidney, lung and spleen was reported between 1 and 7 days (39;74). Despite the observation that viral DNA was present in the salivary glands in dogs following intra-prostatic administration of HAdV-5 (74), in humans shedding via saliva could not be detected (72). In addition, shedding did not occur via faeces or ear and nasal swabs (72;75). Excretion of viral particles via urine on the other hand was observed in humans up to 32 days following injection depending on the dose (54;75).

3.2.1.6 Brain administration
In general, no viral DNA was detected in whole blood or plasma samples following administration of HAdV-5 to the brain in humans (69;70;76-78). Only in one study, where an HAdV-5 viral vector was injected into the wound bed after resection of a brain tumour, adenovirus was detected in plasma samples of 2 patients 3 days after administration (79). However, in several other studies, samples of earlier (day 1-2) as well as later time points (day 5-1 month) were negative, as were samples of day 3 itself (69;70;76-78). As mentioned earlier, this indicates that HAdV-5 is not transported over the blood-brain barrier and most likely the biodistribution to the systemic circulation in the study by Di Pasquale and Chiorini (79)is due to the surgery and damage to the blood-brain-barrier. Data regarding spreading to peripheral organs are not available, but can be expected to be negative, considering the absence of viral DNA in the bloodstream. Conform the lack of viral particles in the bloodstream, no indications of shedding were found. Samples of urine, faeces, sputum and nasal swabs were tested several times from

24 hours until 1 month following administration of HAdV-5 viral vectors to the brain of human patients and no presence of viral vector particles was detected (69;70;76-78).

3.2.1.7 Intra-muscular administration

Biodistribution of viral vector particles following intra-muscular administration of HAdV-5 depends on the muscle group that is injected. Whereas viral genome copies could be detected in venous blood 1 hour following intra-coronary injection of doses larger than $2*10^9$ vp in humans, probably via direct diffusion to the pulmonary arteries, no evidence of viral spreading to blood was observed 2 days up to 12 weeks following injection of up to $2.9*10^{10}$ vp in muscles of the leg (80;81). One day after intra-coronary injection of an HAdV-5 viral vector in pigs, viral DNA was found in liver, lung and spleen (51). Nevertheless, in another study, no shedding via faeces or urine could be detected in pigs on day 2 or 7 (82). In addition, adenovirus was not detected in urine, faeces and semen samples or throat swabs of human subjects injected either intra-coronary or in leg muscles up to 12 weeks after viral delivery (80;81).

3.2.1.8 Inhalatory and intranasal-bronchial administration

Following administration of $1*10^7 – 9.4*10^8$ pfu to the nose and lungs of cystic fibrosis patients, blood samples were regularly analysed for the presence of viral DNA. Up to 28 days after administration, no viral DNA was detected (83). Results on shedding appear to depend on the exact area of administration. Incidental findings of shedding via faeces and urine are reported following nasal administration of HAdV-5 viral particles.

Wild type adenovirus shedding in urine was measured, but no recombinant adenovirus shedding was detected (84). Following delivery of viral vector to the lower airways of cystic fibrosis patients, no evidence of shedding via urine, faeces or sputum could be found within 2 days (85). When virus was delivered to both the nasal and bronchial epithelium, the presence of viral DNA was found in saliva (only within 1 week), nasal brush (within 2 weeks) and bronchial fluid samples (up to 3 weeks) (83). Extensive data on biodistribution following inhalatory administration of HAdV-5 are lacking. Occasional presence of viral vector in the tonsils was reported in humans after 1 or 4 days (83). In addition, viral DNA was found in the lungs of mice 10 minutes up to 24 hours following viral delivery of $1*10^9$ pfu into the trachea (86).

3.2.1.9 Administration via other routes

Ocular administration of HAdV-5 in monkeys resulted in detection of viral DNA in blood from 15 minutes up to 7 days after injection. At day 6, viral DNA was present in bone marrow, eye, heart, kidney, liver and spleen, but not in gonads and lungs. At day 29, viral DNA was still found in the liver and spleen, but not anymore in blood and bone marrow (87). In humans, no shedding via sputum or urine was detected 3 weeks after ocular delivery (88).

When a recombinant HAdV-5 vector was delivered into the submandibular gland of rats, spreading to blood, lung, liver and gonads was observed at doses of $6*10^9$ vp or higher, but no viral DNA was found in these organs for longer than 29 days. Mandibular lymph nodes and injected glands tested positive for up to 92 days. Brain, kidney, intestines, buccal oral mucosa, palatal oral mucosa and tongue remained negative for viral DNA, and only occasional (non dose-related) positive results were found in heart and mucosa of the floor-of-mouth (89;90). Shedding data were not present.

After viral delivery of HAdV-5 vectors at a dose of $2.5*10^{10}$ pfu to the para-aortic lymph node, spine or ileum in human subjects, infectious virus was found in blood samples at day 2 and sometimes day 3. At a dose of $2.5*10^8$ pfu, however, no viable virus was detected in all 3 administration routes. Shedding in urine was not detected 2-10 days after injection (54).

One day after administration of HAdV-5 viral particles into the bladder of mice, viral DNA could be detected in the bladder, liver, heart, gonads, lung, ureter, kidneys and adrenal gland (50).

In one study, the distribution following in utero administration of an HAdV-5-LacZ viral vector was analysed. Seventy-two hours after injection of the viral vector into the amniotic fluid, transgene expression was observed in the digestive epithelium, lungs and surface of the skin, whereas administration to the intra-peritoneal space of the foetus resulted in positive X-gal staining in the peritoneum and the upper part of the digestive tract. However, when the virus was delivered to the placental parenchyma, β-galactosidase expression could not be detected in the foetal organs (91). In the same study, the HAdV-5-LacZ viral vector was distributed via the umbilical vein to foetal guinea pigs. After 24 hours, foetuses were taken out by caesarean section and analysed for virus distribution. Presence of viral genomic DNA was found in liver, heart and spleen, and to a lesser extent also in the adrenals, brainstem, kidney, small intestine, placenta, lung and muscle, but not in the cerebral cortex (91).

3.2.2 Shedding per excretion route

3.2.2.1 Urine

Shedding in urine was detected when HAdV-5 was administered intravenously (46), intra-tumorally (59;61) and intra-prostatical (54;75). Shedding in urine or urethral swabs could not be detected after intraperitoneal (55;56), intra-pleural (57), intra-muscular (80-82), inhalatory/intranasal-bronchial (85) or ocular (88) administration of HAdV-5, or when HAdV-5 was administered directly into the brain (70;76-78), para-aortic lymph node (54), spine (54) or ileum (54). Urine has not been tested for the presence of HAdV-5 after dermal administration or administration in the salivary gland, bladder or maternal urine after administration to the foetus.

The presence of HAdV-5 in urine and plasma (46) suggests the possible presence of HAdV-5 in kidney. This is confirmed in several pre-clinical studies that reported an HAdV-5 signal in kidney after intravenous delivery in mice (39;42). In non-clinical studies also the long term presence of HAdV-5 in plasma is confirmed. One study indicated that the expression of the cloned transgene in serum is gradually decreasing and finally undetectable at 63 days after administration (42). This is in agreement with the observation that at day 70 no HAdV-5 could be detected in kidney (42). It is therefore expected that also shedding in urine after day 70 could not have been detected, although this has not been investigated.

After intra-tumoral administration of HAdV-5 ($1*10^6$ to $1*10^{11}$ pfu) in patients with non-small-cell lung cancer, shedding in urine is reported within 24 hours of injection in all patients (n=24). After 9 days, HAdV-5 could not be detected in urine (59). Shedding in urine was also reported by Fujiwara et al. (2006), after intra-tumoral administration of the same tumour type ($1*10^9$ to $1*10^{10}$ pfu) (61). However, these authors detected a positive signal on days 3 to 14 in 3 out of 12 patients. In this study, no shedding was detected on days 1 and 2 and all patients tested negative on day 15. After day 15, no tests for shedding were performed (61). Kidney samples obtained from the 2 deceased patients on day 25 and 151 were negative (61). One of these patients did show shedding in urine until day 14. The negative kidney signals are consistent with the fact that after day 14 no shedding in urine was observed anymore. Together, the data suggest that the vector is cleared from the kidney within 15 days following intra-tumoral administration of HAdV-5. In addition, no HAdV-5 was detected in urine 2 days following intra-tumoral administration to the same tumour type with a vector dose of ($1*10^7$ to $1*10^9$ pfu) (60). Administration into other tumour types demonstrated the absence of shedding in urine, e.g. administration into liver tumours (63), mammary tumours (64), melanoma (64;65) and soft tissue sarcoma (67).

Intra-prostatic administration of HAdV-5 results in shedding of HAdV-5 via urine (54;75). Shedding via urine was observed when HAdV-5 was injected in the prostatic fossa (54) or into the prostate gland (75). When patients received HAdV-5 by injection in the prostatic fossa, no HAdV-5 material was detected in blood serum (54). In addition, the presence of replicating adenovirus in blood was not detected following injection into the prostate gland (75). Also kidney remained negative for HAdV-5

material in a study investigating the biodistribution of intra-prostatic injected HAdV-5 in mice, but showed biodistribution to the liver (39).

3.2.2.2 Faeces

Shedding of HAdV-5 via faeces has been observed following several administration routes. Following intravenous injection of an HAdV-5 viral vector, shedding was detected in the faeces of 1 out of 6 patients, somewhere between 1 and 28 days after injection (46). In addition, following intravenous injection in mice, HAdV-5 was detected in bile from 10 minutes up to 24 hours after delivery of the virus (84), indicating that the virus is shed via faeces. Intra-tumoral injection of low doses of HAdV-5 ($\leq 10^7$ pfu) did not result in faecal shedding 1-2 days after injection in patients (60;63), whereas in the same period, at doses $\geq 10^8$ pfu, viral DNA was detected in stool samples (60). Similarly, faecal shedding following the inhalatory administration route seems to be dose-dependent, with shedding only observed at doses of 10^{10} pfu (84;85). In addition, data indicate that faecal shedding is time-dependent, and only occurs (at doses of 10^{10} pfu) in the first 2 days after nasal administration, but not afterwards (84).

Shedding of HAdV-5 via faeces could not be detected when administered via the intraperitoneal (55;56), intra-pleural (57), intra-prostatic (72), or intra-muscular route (81;82) or after administration to the brain (76;77) and was not analysed following the other administration routes (dermal, ocular, salivary gland, para-aortic lymph node, spine, ileum, bladder and in the mother after foetal administration).

3.2.2.3 Mouth and nose secreta

Shedding of HAdV-5 via mouth and/or nose has not been investigated systematically. Indications for shedding of HAdV-5 via mouth and nose were reported following intra-tumoral (59-62;68), inhalatory and intranasal administration (83). Shedding via nose and mouth after intravenous, intra-prostatic, brain, intra-muscular and ocular administration has not been observed. No data is published indicating the presence or absence of shedding via mouth and nose regarding dermal administration and administration directly to the salivary gland, para-aortic lymph node, spine, ileum and bladder.

Following intra-tumoral administration to different tumour types like lung cancer tumours (59-62) and head and neck squamous cell carcinoma (68), HAdV-5 material was detected in several mouth and nose secreta samples which might indicate HAdV-5 shedding via mouth and/or nose. Positively tested samples were sputum (59;60;68), bronchial fluid (60), saliva (59;68), throat swabs (60) and gargle (61). In a clinical study where HAdV-5 was administered intra-tumorally in lung cancer tumours, sputum and saliva tested positive for HAdV-5 within the first 24 hours of injection in all tested patients (n=18) that received dose levels between $1*10^6$ to $1*10^{11}$ pfu. Nine days after injection no positive results were obtained. Testing was based on a CPE assay, meaning no infectious HAdV-5 particles were present (59). In another study, 21 lung cancer patients were injected intra-tumoral with 10^7 to 10^9 pfu HAdV-5. Positive sputum samples (days 1 to 60) were detected by PCR in 15 patients. With a CPE assay, the number of positively tested samples was strongly reduced compared to the previous study; only two HAdV-5 positive samples on day 1 and 2 were found (60). In this study, throat swabs were also positive between days 1 and 11 in six out of twenty-one patients. A CPE test performed on these samples did not reveal the presence of HAdV-5 infectious particles. Bronchial fluid samples collected (up to 90 days) were HAdV-5 positive (PCR) on days 1 (20 patients) to 90 (4 patients). With a CPE assay, HAdV-5 positive bronchial fluid was only detected on day 0 just after administration (60). In a recent study, HAdV-5 was detected by PCR in 29 of 39 gargle samples obtained 1 day after vector injection, regardless of dose level (10^9 to 10^{11} pfu) and declined to undetectable levels within 15 days for most patients. In total, 90 gargle samples (14.4%) were positive for vector DNA (61). Infectious HAdV-5 was also detected in sputum and/or saliva samples of patients who received HAdV-5 intra-tumorally injected in head and neck squamous cell carcinomas (10^{11} pfu) (68). At lower doses all samples remained negative. The highest titer found was 10^6 pfu per 0.5 ml sputum or saliva.

Shedding via nose and mouth secreta has also been reported following inhalatory and intranasal administration used for treatment of cystic fibrosis patients with dose levels ranging from 10^5 to $5.4*10^8$ pfu (83). CPE testing did not reveal the presence of HAdV-5 in cultures derived from nasal brush, bronchial brush or saliva. Using PCR, HAdV-5 was detected in nasal brush (up to 21 days post administration), bronchial brush (up to 14 days post administration) and in saliva (up to 14 days post administration) (83).
HAdV-5 shedding via nose and mouth has not been reported following intra-tumoral administration of HAdV-5 to other tumour types like metastatic breast cancer tumours (64), melanoma (64), liver cancer tumours (63), soft tissue sarcomas of the extremity (67) and recurrent malignant gliomas (70). In these studies no HAdV-5 was detected in throat swabs (63;64), sputum (67) and nasal swabs (70).

3.2.2.4 Semen

The presence of HAdV-5 material was detected in sperm of rabbits 2 weeks following intravenous administration, although according to the authors, this was probably due to the presence of (positive) blood in the sample (44). In addition, following intravenous administration distribution of HAdV-5 could also be found in the testes of rabbits and baboons (44;48), as well as in the prostate and ureter of mice (39;50). However, adenovirus was not detected in sperm 8 weeks following intra-muscular administration in human, but biodistribution to organs involved in the production or excretion of semen were not analysed (80). For the other administration routes described in this review, shedding via semen was not analysed.

3.2.2.5 Various smaller excretion routes

After dermal application, only local distribution was found and shedding was observed via the wound bed (71). No other excreta were measured. The other HAdV-5 administration routes did not investigate biodistribution to the skin or shedding via a wound bed or skin.
Shedding via ear swabs was only studied by Herman et al (1999) and did not occur within 4 days after intra-prostatic administration of $1*10^8$ to $1*10^{11}$ iu (75). However, the detection method was based on culturing for wild type adenovirus.
After intravenous administration of $9.5*10^{11}$ vp HAdV-5 to rabbit, the eyes were positive on day 1 using quantitative PCR. No other biodistribution studies investigated the distribution to the eye or the shedding via lachrymal fluid.

3.3 Results AAV2

Biodistribution and shedding of AAV2 viral vectors are dependent on the route of administration. In table 3 the biodistribution and shedding routes of AAV2 viral vectors following various administration routes are described. Detailed information on the biodistribution and shedding per experiment can be found in Appendices A (Table A.16 to A.24) and B.

Table 3. Biodistribution and shedding of AAV2

Administration route	Biodistribution	Shedding
intravenous	*positive*: plasma, brain, liver, lung, spleen, kidney, gonads, heart, bladder, muscle, GI-tract, salivary gland, lymph node, retina, tonsil, bone marrow, gallbladder, thyroid, pancreas, skin, thymus, trachea, adrenal gland and peripheral blood mononuclear cells *negative*: spinal cord	*positive*: saliva, urine and semen
intraperitoneal	*positive*: abdominal tissue, diaphragm and intercostal muscle *negative*: heart, liver, lung, kidney, spleen, other muscles and testis	no data available
intra-muscular	*positive:* serum, heart, kidney, liver, lung, spleen, gonads, injected muscle, lymph node and epididymal fluid *negative:* intestine, other muscle, adrenal gland, brain, blood cells, thymus, thyroid and spinal cord	*positive:* saliva, urine, semen, faeces, lachrymal swab and nasal swab *negative:* stool
inhalatory and intranasal-bronchial	*positive:* blood	*positive:* sputum *negative:* urine, stool, BAL, nasal swab and nose secretion
ocular	*positive:* diaphragm, heart, lymph node, kidney, eye, visual cortex and LGN *negative:* gonads, jejunum, liver, lung, muscle, pancreas, spleen, cerebellum and thalamus	no data available
salivary gland	*positive:* salivary gland, liver, spleen and testis *negative:* heart, lung, kidney and lymph node	no data available

Table 3. Biodistribution and shedding of AAV2 (continued)

Administration route	Biodistribution	Shedding
intra-cochlear and intra-articular	*positive:* cerebellum, cochlea *negative:* serum, cortex, heart, kidney, liver and lung	no data available
intra-cerebral lateral ventricle	*positive:* brain, meninges and pial surface	no data available
isolated limb perfusion	*positive:* serum and perfused muscle *negative:* kidney, liver, lung, control muscle, gonads and spleen	no data available

LGN = lateral geniculate nucleus
BAL = broncho-alveolar lavage
GI-tract = gastrointestinal tract

3.3.1 Biodistribution and shedding per administration route

3.3.1.1 Intravenous administration

In a clinical trial for the treatment of haemophilia, AAV2 was delivered to the liver via the hepatic artery which resulted in unexpected transient vector dissemination to the semen which was detected by PCR. Results from serum and urine suggested that clearance was dose dependent. Urine was the first body fluid to clear. There was no association between dose and time for the clearance of semen. The last positive semen samples in the lowest dose cohort were at 12 weeks, whereas the last positive samples in the five subjects in the two higher- dose cohorts occurred at a mean of ~5 weeks. Age of the subject seemed to be the best predictor for the time needed for the semen to clear, with young men on average clearing more quickly than older men. Semen fractionation, carried out for a single subject, showed no evidence of vector sequences in motile sperm. Peripheral blood mononuclear cells showed the longest duration of positive results of any body fluid or tissue analysed (92). In pre-clinical studies following administration of AAV2 to the hepatic artery, biodistribution to the gonads was analysed in rats after 50 and 92 days. A dose-dependent response was observed; 50 days after injection the percentage of animals with a positive signal in the gonads increased with increasing doses of AAV2 and after 92 days positive results were only observed in rats that had received the highest dose ($1*10^{13}$ vp/kg) (93). The liver was positive for at least 7 weeks in rats treated with $5*10^{12}$ vp/kg (94). However, in this study no other organs or shedding were investigated. Also in rabbit, administration of AAV2 to the hepatic artery resulted in detectable vector sequences after more than 18-20 months in the gonads of animals that were administered a dose of $1*10^{12}$ or $1*10^{13}$ vp/kg, but not in animals that received $1*10^{11}$ vp/kg. The liver was positive for more than 1 year after injection in all dosage groups. At 4 days after administration of the virus, infectious particles were detected in semen, but not anymore after 7 and 14 days. The presence of viral DNA however, was found up to 6, 8 or 13 weeks after doses of $1*10^{11}$, $1*10^{12}$ and $1*10^{13}$ vp/kg, respectively (95). In mice administered AAV2 ($2.1*10^{10}$-$8.4*10^{10}$ vp) via the portal vein or via tail vein infusion, the liver remained positive for 49 days, while the muscle and spleen were negative within 7 days (96). In mice treated with $2*10^{11}$ vp, the brain, heart, kidney, liver, lung and spleen (investigated organs) were still positive at 6 weeks after administration (97). Biodistribution to the gonads or shedding in the semen was not detected between 7 and 90 days after injection ($3.7*10^{12}$ or $7*10^{12}$ vp/kg) in dogs into the hepatic artery (93).

Four biodistribution studies have been performed in monkeys (98-101). Following administration of AAV2 into the hepatic artery or portal vein of Macaques, viral DNA was found in plasma after 3 and 6 days, but not after 8 days. In addition, after 11 months, viral DNA was found in the liver and spleen, but not in the kidneys or gonads (100). A previous study by Nathwani *et al.* (2001) showed that the heart, liver and spleen were also positive for viral genome particles after 22 weeks of administration (98). However, kidney and lung were negative at 22 weeks. Mori *et al.* (2006) performed an extensive study concerning the biodistribution of AAV2 following injection of several doses in the femoral vein of Cynomolgus monkeys (101). Doses varied from $1*10^{10}$ to $2.5*10^{11}$ vp/kg and follow up time after viral injection varied from 2 days to 7 months. Colon, spleen and several lymph nodes (axillary, hilar, iliac and mesenteric) tested positive for viral DNA for all doses and time points. In addition, liver, tonsil, bladder and inguinal lymph nodes were positive for all doses and time points, except 3 months after injection of the lowest dose. At doses of $2.5*10^{10}$ or higher, viral DNA was detected in bone marrow, gall bladder, lung and pancreas for 2 days up to 5 months after injection. At such doses viral DNA was also present in heart and kidney after 3 months (negative on day 2) and in the trachea and cerebrum 2 days and 3 months after injection. Adrenals and muscle were only observed to be positive after doses of $1*10^{11}$ vp/kg or higher (3 and 5 months) and retina was found positive (7 months) after the injection of $1*10^{10}$ vp/kg. Occasionally, not dose- and/or time-related positive findings were obtained in the cerebellum, gonads, ileum, oesophagus, stomach, salivary glands, skin, thymus and thyroid. In the jejunum and spinal cord, evidence for the presence of viral particles was never detected (101). Evidence for shedding in monkeys was only observed in saliva and urine, up to 3 and 6 days respectively, following administration to the hepatic artery or portal vein (100). Other shedding routes were not analysed.

3.3.1.2 Intraperitoneal administration

Only one study reported the biodistribution after intraperitoneal administration of AAV2 (102). Two months after viral delivery ($2*10^{11}$ vp) in neonatal mice, viral DNA was found in the intercostal muscles, abdominal tissue and diaphragm, but not in the heart, kidney, liver, lung, spleen, testis and other muscle tissue (triceps and masseter) (102). Shedding was not studied, but data indicate that AAV2 remains locally situated.

3.3.1.3 Intra-muscular administration

Biodistribution of viral vector particles following intra-muscular administration of AAV depends on the muscle group that is injected. After intra-myocardial administration of AAV2 ($4*10^{11}$ vp) to rat, biodistribution of AAV2 was observed to the testes, heart, kidney and liver, but not to the spleen (103). Blood or plasma was not studied, but because AAV2 vectors were measured outside the heart, it is expected that the viral vector is transported throughout the body via the systemic circulation.

Two limited biodistribution studies were performed in mice with AAV2 injections into muscle of the leg. AAV2 was present in the liver after 56 days after a dosage of $1*10^{11}$ vp and in the gonads up to 91 days after dosages of $1.7*10^{11}$ and $1.7*10^{12}$ vp/kg (93;104). In another biodistribution study administering $5*10^{10}$ vp in mice, it was shown that after 22 weeks the measured organs were still positive for viral genomic copies (98).

One very limited biodistribution study after intra-muscular administration was performed in rat by Arruda et al., 2001 ($2.8*10^{11}$ and $2.8*10^{13}$ vp/kg) (93). Epididymal effluent was positive at 15 days after injection into the leg muscle. No other tissues were studied.

Arruda et al., 2001 also studied the biodistribution of AAV2 in rabbits (93). Serum was positive up to 48 hours and negative at 7 days and gonads were positive up to 90 days. However, semen was negative throughout the study, indicating that in contrast to in rats, semen is not a shedding route of AAV2 in rabbit.

Several biodistribution studies and one shedding study were performed in dogs after intra-muscular administration to the hind legs or all four legs (93;105-108). After administration of $2.5*10^{12}$ vp, only the injected muscle and regional lymph node were positive after 10 weeks and not the adrenal gland, brain, kidney, liver, lung, control muscle and testis (106). Similar results were found by Chao *et al.* after administration of $1.2*10^{13}$ vp to all four legs and by Arruda *et al.* after isolated limb perfusion and intra-muscular administration of $2*10^{12}$ vp/kg (105;107). Serum was positive up to 5 days after isolated limb perfusion and intra-muscular administration of $2*10^{12}$ vp/kg (107). After 8 weeks, 12 weeks and 33 weeks, the injected or perfused muscle was still positive (105;107;108). After 5 days, serum was negative and also the gonads, kidney, liver, lung and control muscle were negative at 8 weeks (107). Also the peripheral blood mononucleated cells, brain, intestine, lymph nodes, spinal cord and spleen were negative after 33 weeks (105). In addition, semen was negative up to 16 months in one study (93). In monkeys (Cynomolgus and Rhesus), a dosage of $5*10^{8}$–10^{10} iu/kg into the arms and legs resulted in biodistribution to the liver, lymph nodes and muscle after 8 to 18 months (109). Serum contained viral particles up to 6 days and was negative from day 7. Brain, gonads, heart, intestine, kidney, lung, and spleen were negative for AAV2 after 8 to 18 months. Song *et al.* also found negative gonads after intra-muscular administration of $5*10^{12}$ vp/kg to Baboons after 4 months (110). Shedding was observed in faeces (up to 6 days), saliva (up to 2 days) and urine (up to 6 days). Also lachrymal swabs and nasal swabs were positive for AAV2 up to 6 days. These results indicate that AAV2 can be shed via several routes after intra-muscular administration to monkeys.
In two studies, the biodistribution and shedding of AAV2 after intra-muscular administration in humans was investigated. After a dosage of $1.4*10^{13}$-$7*10^{14}$ vp, shedding was observed in saliva (up to 2 days and then negative) and urine (up to 1 day and then negative) (25). No shedding via semen was observed. Serum was positive up to 12 weeks and negative at 14 and 16 weeks (end of study). Kay *et al.* showed after administration of $2*10^{11}$ vp/kg ($1.4*10^{13}$ vp for an average man) that AAV2 is shed via saliva (24 hours and then negative) and urine (24 hours and then negative) (111). Semen and stool were negative. Serum was positive up to 48 hours, but negative after 1 year. The injected muscle remained positive for 1 year.

3.3.1.4 Inhalatory and intranasal-bronchial administration

Following delivery of AAV2 ($1*10^{10}$ - $1*10^{13}$ drp) to the lungs of patients with cystic fibrosis via aerosol nebulisation, viral genome copies were detected in the plasma of 1 patient injected with 10^{10} particles 1 day after infection. Stool and urine samples of all patients remained negative for shedding during the 90 days follow up. Sputum samples of patients injected with 10^{12} particles were positive only on day 1. In addition, positive sputum samples of patients injected with 10^{13} particles were detected on day 1 and 7, but not anymore on day 14 or 30 (112). In another study where 10^{13} particles were administered to cystic fibrosis patients via aerosolisation, shedding in sputum was observed up to 150 days (113). However, in a study by Wagner *et al.* in cystic fibrosis patients, all shedding samples (stool, urine and nose secretion) were negative from 2 days until 34 days for viral genome copies (114). Also blood was negative on day 1 and 7.
Shedding routes were analysed following administration of AAV2 particles to the nose and the superior segment of the lower lobe of the lung in cystic fibrosis patients. Although sputum tested positive for viral shedding in 1 patient on day 1, sputum from other patients and samples from urine, stool, broncho-alveolar lavage and nasal swabs from all patients were negative between day 1 and 30 (115).

3.3.1.5 Other administration routes

Delivery of an AAV2 vector to the retina of dogs resulted in the presence of viral DNA in the diaphragm, heart, mandibular lymph node and optic nerve and chiasm 3 months after injection. Other major organs (liver, lung, kidney, pancreas, spleen and gonads) as well as other lymph nodes and parts of the visual system remained negative (116). Also in monkeys no major organs showed presence of AAV after subretinal administration. In addition, tested areas in the brain (cerebellum and thalamus)

were negative. Only in the LGN viral DNA was detected 3 months after administration. In comparison, following intra-vitreous administration, the only positive result was found in lung tissue. When analysed 1 week following subretinal injection, however, the presence of viral DNA was found in the kidneys, mandibular and pre-auricular lymph nodes, and several parts of the visual system (LGN, optic nerve, optic tract, retina, superior colliculus and visual cortex) (99).
Following submandibular administration of AAV2 in mice, biodistribution was found to the liver, spleen and testes 8 weeks after delivery (117). When viral biodistribution was studied in monkeys, six months after administration of 10^{10} or 10^{11} vp to the parotid gland, high amounts of viral vector were still found in the targeted gland, whereas other salivary glands, cervical lymph nodes and major organs (heart, kidney, liver, lung, spleen and gonads) were negative (118). Shedding was not studied following delivery of AAV2 to the salivary glands.
In only two studies, the administration into sections of the inner ear (cochlea and articular) was investigated. After administration into the cochlea of guinea pigs, the vector was distributed to the cerebellum and the not treated cochlea (119). However, after 2 and 4 weeks the cortex, heart, kidney, liver and lung were negative. In addition, after 21 days serum was negative in rabbit treated with $1.5*10^{12}$ iu into the articular (120).
After intra-cerebral lateral ventricle administration of $1.8*10^{10}$ vp in mice, various sections of the brain, the meninges and pial surface were positive indicating distribution within the brain and the spinal cord (121). Brain administration to neonatal animals was not included, because only the distribution in the brain was studied and not distribution outside the brain.
After isolated limb perfusion in dog, serum was positive for the first 5 days and then negative (107). After 8 weeks only the perfused muscle remained positive and the other investigated organs (kidney, liver, lung, ovary, and spleen) were negative. No shedding data was available.

3.3.2 Shedding per excretion route

3.3.2.1 Urine

Shedding of AAV2 via urine has been reported when the vector was administered by intravenous (92;100;101) or intra-muscular injection (25;109;111). Urine samples were negative after inhalatory and intranasal-bronchial administration (112;114;115). The presence of AAV2 in urine has not been tested when the vector was administered using other administration routes.
Following intra-muscular administration of AAV2, several reports describe an AAV2 positive signal in urine. Urine samples from 3 out of 8 patients who received AAV2 via intra-muscular administration ($2*10^{11}$ – $1.8*10^{12}$ vector genomes/kg) tested positive for vector sequences up to 24 hours but not thereafter (25). This is in agreement with the observations described by Kay *et al.* who reported positive urine samples at 24 hours, but not thereafter following administration of $2*10^{11}$ vp/kg (111). In all patients plasma was tested positive for the presence of AAV2 but in most cases not anymore after day 7 (25). In other studies, serum samples were negative after 48 hours (111). These observations are in agreement with reports on AAV2 shedding in urine in Rhesus and Cynomolgus monkeys where AAV2 was detected in urine samples collected 6 hours to 6 days post administration ($5*10^{8}$ to $1*10^{10}$ iu/kg) (109).
Following delivery of AAV2 via aerosol nebulisation to the lungs of patients with cystic fibrosis, urine samples of all patients remained negative for shedding during the 90 days follow up (112). In agreement with these findings also Wagner et al. (1999) did not detect AAV2 shedding in urine measured 2 days until 34 days after administration (114). In addition Flotte et al. (2003) also did not detect AAV2 in urine samples collected between day 1 and 30 after administration (115).

3.3.2.2 Faeces

In Cynomolgus and Rhesus monkeys, shedding was observed in faeces in the first 6 days after administration of $5.0*10^{8}$-$1.0*10^{10}$ iu/kg (109). However, in humans treated with $2.0*10^{11}$ vp/kg, faeces were negative for the period investigated (24 h to 59 days) (111). In addition, human stool

samples were negative from 1 to 90 days after inhalatory and intranasal-bronchial administration (112;114;115). No other studies investigated the shedding via faeces of AAV2.

3.3.2.3 Mouth and nose secreta

Saliva from monkeys (Macaque) was positive for viral DNA on day 3 and negative on days 6 and 8 after intra-arterial administration to the hepatic artery of $4*10^{12}$ vp/kg (for an average Macaque a dose of $2*10^{13}$ vp) (100). Also in saliva from Rhesus and Cynomolgus monkeys treated with $5*10^{8}$-$1*10^{12}$ iu/kg (average dose of $2.5*10^{9}$–$5*10^{10}$ iu) in leg muscles, viral DNA was detected for the first 2 days but not thereafter (109). In human haemophilia patients, saliva was also positive for viral DNA after intra-muscular administration to leg muscles ($1.4*10^{13}$-$7.0*10^{14}$ vp) up to 2 days and then negative up to 12 weeks, with only one positive sample at 2 weeks (25). After *in vastus lateralis* administration of $2*10^{11}$ vp/kg (average of $1.4*10^{13}$ vp) in human haemophilia patients, saliva was positive at 24 hours and negative at 48 hours (111). In addition, sputum was positive at 150 days after inhalatory administration of $1*10^{13}$ drp to human cystic fibrosis patients (113). However, in two other studies different shedding results were obtained in human cystic fibrosis patients. In one study, patients were treated intranasal-bronchially with $3*10^{1}$-$1*10^{9}$ ru and sputum was positive on day 1 for viral DNA and negative on day 7 to 30 (115). In the other study in patients treated inhalatory with 10^{10}-10^{13} drp, sputum was positive from day 1 and 7 and negative on day 14 and 30 using an infectious assay (112).

Shedding via the nose (nose swab and nasal secretion) was observed in monkeys up to 6 days after intra-muscular administration (109), but not on day 1 to 34 after intranasal-bronchial administration to humans (114;115). Also, after administration of $3*10^{1}$-10^{9} ru to human cystic fibrosis patients, BAL obtained from these patients was negative on day 1 to 30 (115). No other studies were performed on shedding via mouth and nose secreta.

3.3.2.4 Semen

Shedding of AAV2 via semen has been reported after intravenous (92;93;95) and intra-muscular (93;103;104) administration. For other administration routes, no data on shedding via semen has been reported.

In a study with adult haemophilia B men, AAV2 delivered to the liver via the hepatic artery resulted in transient vector dissemination to the semen. In these patients also urine samples were tested positive for AAV2 although clearance from urine samples occurred earlier than the clearance of AAV2 via semen. The authors found that clearance from semen was not dose dependent. The last AAV2 positive semen samples were detected by PCR at 12 weeks (92), which is similar to observations in rabbits were AAV2 sequences were detected by PCR up to 13 weeks after administration (95). In the latter study, it was found that the presence of infectious particles did not last for 13 weeks. At 4 days after intravenous administration of AAV2 in rabbits, infectious particles were detected in semen, but at 7-14 days no infectious AAV2 particles were detected (95). Also in rats positive semen samples were found following intra-muscular delivery of AAV2 (93;122).In contrast with the observed presence of AAV2 in humans, rats and rabbits; no AAV2 in semen was detected using PCR between 7 and 90 days after injection of $3.7*10^{12}$ or $7*10^{12}$ vp/kg into the hepatic artery or muscle of dogs (93).

3.3.2.5 Various smaller excretion routes

A lachrymal swab from Rhesus and Cynomolgus monkeys was positive for 6 hours to 6 days after intra-muscular administration of $5*10^{8}$ – $1*10^{10}$ iu/kg (109). From day 7, the lachrymal swab was negative.

After intra-cochlear administration, the injected and control cochlea were positive during the sampling period of 4 weeks (120). However, shedding via the ear was not investigated.

The skin in monkey was positive at 3 month after intravenous administration of $2.5*10^{10}$ vp/kg (122). However, shedding via the skin or a wound bed was not investigated.

3.4 Quantitative modelling

In the literature search publications were found dealing with the modelling of viral vectors, viruses or vaccines. Several mathematical models concerning viral vectors exist, but they suffer a more general applicability and are not suitable to model the kinetics of HAdV-5 and AAV2 (123-137). Transport from plasma to a cell kernel of some tissue at the cellular level or the behaviour at a higher level than cellular, but for one specific vector variant only, was described.
The review of Ledley and Ledley concerns the pharmacokinetics of gene products, but not the pharmacokinetics of the gene delivery system (138). It introduces one-compartment models for the kinetics of gene products after application of a gene therapy (viral vectors, DNA-plasmids) that involves permanent residence (and thus production) in a cell, non-permanent residence in a cell and permanent residence in cell, but where effective gene expression can be controlled (e.g. by a gene expression inducing molecule that is applied as a drug). The authors remark that '*there have been few detailed studies of the parameters affecting the kinetics of the gene or gene product or the quantitative models that describe these events. There are no reports of pharmacokinetic studies in humans*'. Also, the authors, referring to their own modelling contribution to the literature, remark that '*most of the assumptions incorporated in the models are purely theoretical*'. In addition, their models do not consider biodistribution or explicit routes of elimination. The kinetic processes involved in the biokinetics of transgenes that are described are rather qualitative of nature. The transgene DNA is given more attention than viral vectors. Their main conclusion is that '*bioactive gene product is determined by a series of intrinsic kinetic processes that remain incompletely understood or experimentally investigated*'.

4 Discussion

This report presents an overview on published spreading data obtained from non-clinical and clinical trials using replication-deficient gene therapy viral vectors derived from HAdV-5 and AAV2. Replication of viral vectors in the treated subject might affect the spreading potential; therefore only data obtained with replication-deficient vectors are included in this study. The reviewed literature data clearly show that the route of shedding of HAdV-5 as well as AAV2 viral vectors depends on the route of administration. However, it is difficult to compare studies. First of all, viral titres vary among studies, due to various ways of expression, and are difficult to put side by side. Furthermore, different analysis techniques and time points of sampling are used. In addition, often biodistribution and shedding have been studied for only a selective group of organs and/or excreta, which varies between the different studies. One has to keep in mind that negative results do not necessarily indicate that biodistribution and shedding do not occur, because the limit of detection could be too high or the time of sampling could be either too early or too late.
Although non-clinical and clinical trials are included in this report, it is important to realise that spreading profiles can be influenced by the treated species. Studies on the biodistribution and shedding of HAdV-5 and AAV2 were performed in mice, rat, guinea pig, rabbit, dog, monkey (Baboon, Cynomolgus, Rhesus and Macaque), pig and human. Spreading data obtained in an animal model can not be extrapolated completely to a clinical setting in humans. Differences in spreading data found in an animal model compared to spreading observed in a human clinical trial can be due to differences in attachment and entry of viral vectors and due to the presence of neutralising antibodies in humans which are often absent in animals used in non-clinical experiments. The conclusions in this report are only valid for replication-deficient HAdV-5 and AAV2 and can not be extrapolated to other serotypes and other viral vectors.

4.1 Issues in data interpretation

The majority of the investigated literature lacks either biodistribution or shedding data. In case biodistribution or shedding data are provided, these are limited and mostly include only qualitative observations on limited time-points. Biodistribution data can be used to estimate the risk of shedding, because presence of viral vectors in specific organs might be indicative for shedding through specific excreta. For both biodistribution and shedding, detailed information on the experimental design is limited. Furthermore, the experimental design used to collect and analyse data varies considerable (*e.g.* tested tissues/excreta, time schedules for sampling, distribution/shedding assay, detection limits, vector dosage, treated population, treated disease, used vector). It is noted that biodistribution and shedding profiles can also vary within a single study. Some articles showed biodistribution or shedding in only a small subset of the tested individuals. In these cases, it was concluded that biodistribution or shedding is possible.

Biodistribution of gene therapy viral vectors is mostly investigated in animal models. However, in this report focus is on HAdV-5 and AAV2 which both originate from wild type viruses with a human host. Differences between species in receptors needed for viral infection (12;13;17-19), erythrocyte binding (20) or the presence of neutralising antibodies (139;140) in the natural host can influence the biodistribution profile and reduce the predictive value of data obtained in animals for humans. Especially the interaction of the viral vector with the immune system is of great importance. Most humans display innate and adaptive immune responses against the human adenovirus and adeno-associated viral vectors used in gene therapy, while in naïve animals only the adaptive immune response plays a role (141;142). Even in the human population, the innate immunity is very heterogenous (*e.g.* vaccination and environmental exposure). This can lead to variation in biodistribution of the viral vector after administration and can thus influence the shedding potential of the viral vector. Thus, large inter-individual and interspecies variation is observed. In addition, in general biodistribution and shedding data are poorly reported in the literature.

4.2 Quantitative model

Based on the obtained biodistribution and shedding data, a PBK model is proposed in Figure 2. However, at this moment it is not possible to generate kinetic input parameters of HAdV-5 or AAV2 for this PBK model. Nevertheless, such a scheme may be helpful to develop a more qualitative approach, relating site of administration to probable routes of biodistribution and shedding.
The vectors have to migrate from the site of administration to sites where shedding can take place, if shedding during administration is excluded from such a modelling approach. So, it is assumed that the biodistribution of viral vectors after administration could at least help to find which routes of shedding are most probable. For a quantitative modelling approach, there is need for more experimental data on biodistribution and shedding, *e.g.* from sampling blood, tissues (if possible) and excreta at multiple time points from shortly after administration to a period of several weeks or months.

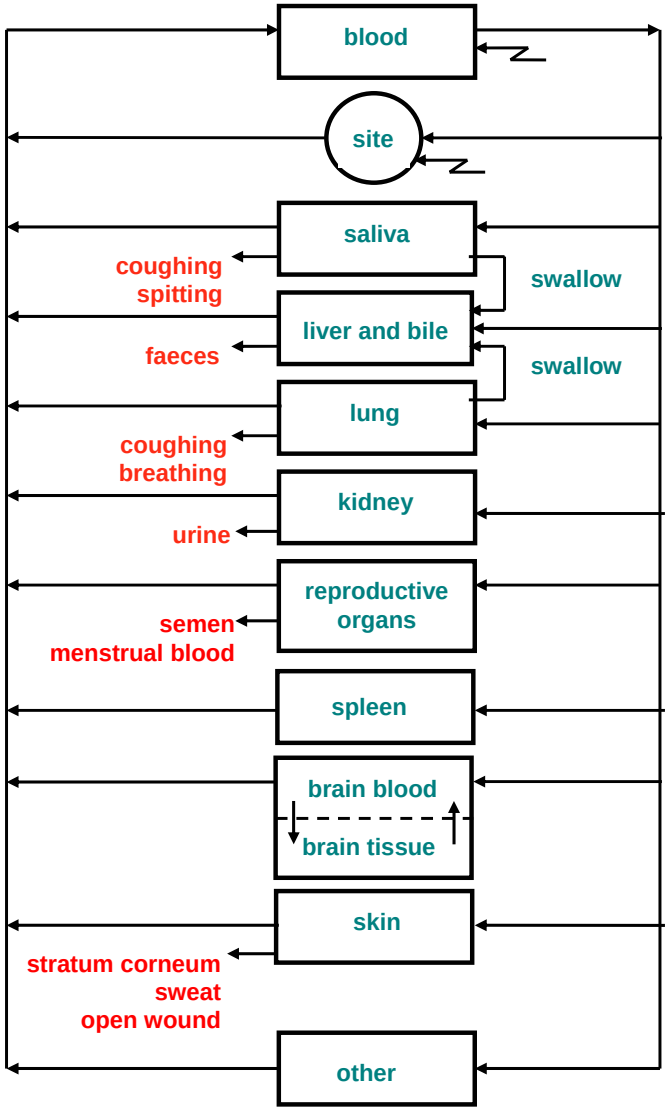

Figure 2. Schematic view of a PBK model that could describe tissue biodistribution and shedding of viral vectors used in gene therapy. Several possible ways for shedding have been incorporated.

4.3 Qualitative model

Based on the PBK model as shown in Figure 2, qualitative models based on the different administration routes were made for HAdV-5 and AAV2, respectively, in order to aid in risk assessment and predicting shedding via various routes.

4.3.1 Adenoviral 5 vector

The CAR receptor, which is involved in the attachment of adenoviral vectors to cells, is present on human erythrocytes but not on mice erythrocytes. This explains the high adenoviral vector concentration in mouse plasma, while human plasma contains a significantly lower (free) adenoviral vector concentration (11-14;20). In addition, the binding of adenoviral vectors to human erythrocytes could result in a decreased tissue biodistribution compared to the biodistribution in mice, since binding to erythrocytes can prevent biodistribution to organs. Therefore, the delivery of adenoviral vectors to human organs might be less efficient than that observed in mice.

4.3.1.1 Shedding of HAdV-5 via urine

A qualitative model for the likelihood of shedding via urine for the different administration routes used for replication-deficient HAdV-5 is shown in Figure 3. In general, there seem to be three possible routes: either via blood and kidney, directly from the bladder or from the prostrate to the ureter. Shedding via urine cannot be excluded in administration routes that result in distribution of HAdV-5 to these organs.

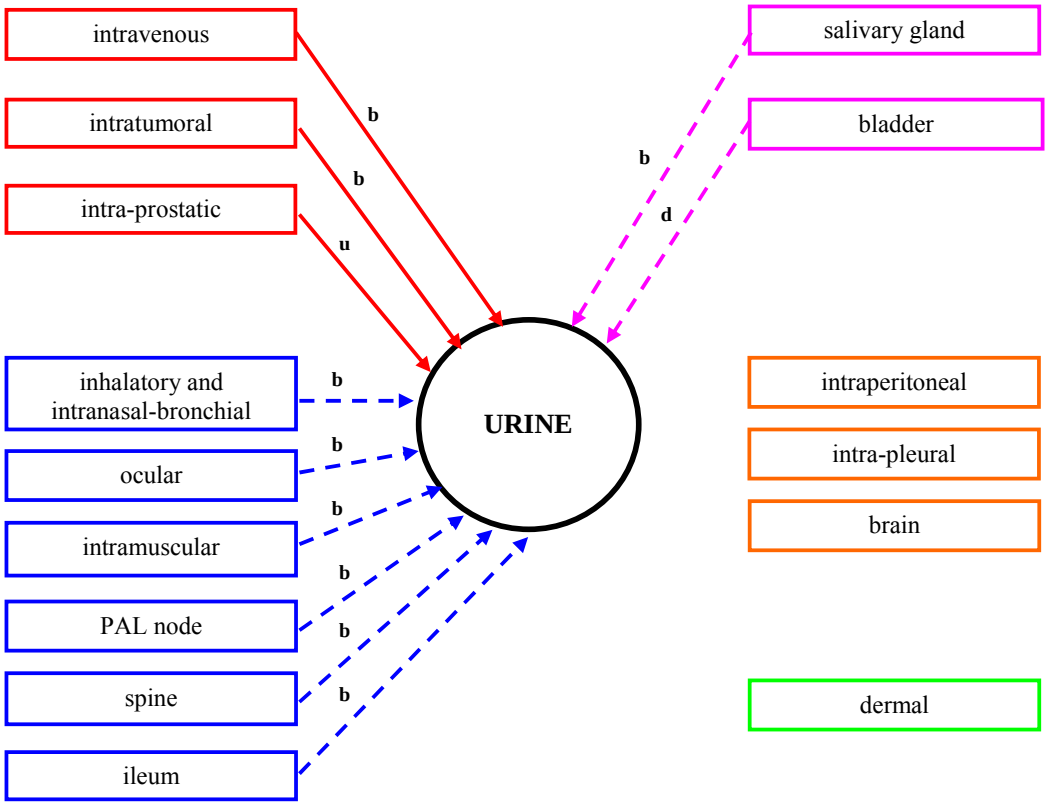

Figure 3. Qualitative model of HAdV-5 shedding via urine from different administration routes. Arrow = shedding via urine; dashed arrow = probable shedding via urine; no arrow = no or unlikely shedding via urine; b = via blood to urine; d = directly; u = via ureter; red = tested and detected in urine and blood; blue = tested and not detected in urine, but detected in blood and/or other organs than site of administration; pink = not tested in urine, but detected in blood and/or other organs than site of administration; orange = tested and not detected in urine, blood or other organs than site of administration; green = not tested in urine and not detected in blood and/or other organs than site of administration.

Intravenous administration of HAdV-5 has been shown to result in biodistribution to kidney in several species (39;42;43;48;50;53;143) and to bladder and prostate in mice (39;50). Thus, the presence of viral material in blood should be considered a forewarning for possible shedding via urine. In addition, shedding via urine after injection of HAdV-5 into the bladder also seems likely, since this administration route in mice results in the presence of HAdV-5 in the bladder as well as in the ureter (50). Following intra-prostatic injection of HAdV-5, the direct route via the ureter is probably responsible for shedding of viral material in urine. The direct contact of the prostate with the ureter may explain why shedding via urine is observed without a positive signal for HAdV-5 in blood or kidney.
Following intra-muscular administration, viral presence in blood, and thus chance of shedding via urine, depends on the muscle injected, with positive blood samples following intra-coronary injection (80), but negative blood samples following injection of leg muscles (81). However, this was only investigated in one study, where an infectious assay with a high limit of detection was used, which could have resulted in false negative results. In addition, the studies mentioned above were performed in different species (humans versus pigs). Therefore, also species differences could account for the contrasting results.
The chance of shedding via urine seems smaller following viral administration to the salivary gland, since kidney remained negative in rats (90). However, the possibility may not be neglected, since blood samples did contain viral DNA (89;90). After inhalatory and intranasal-bronchial administration, shedding via urine was negative (83). Blood samples however were positive and therefore shedding via urine is possible. Also after viral delivery to the spine, ileum or para-aortic lymph node in humans, blood samples were positive (54), indicating the possibility of biodistribution and shedding via urine. Since HAdV-5 can not pass the blood-brain barrier, leakage during administration most likely explains how HAdV-5 reaches the systemic circulation after delivery into the spine (54).
Shedding via urine seems unlikely following dermal administration, where kidneys as well as blood samples remained negative and thus indicate that the viral vector remains locally situated (71). Also after brain administration shedding via urine is unlikely, because it did not result in detectable presence of viral material in blood as long as it was not combined with surgical resection of tumour tissue (70;76;77).

4.3.1.2 Shedding of HAdV-5 via faeces

Shedding via faeces can occur by two possible ways; either via blood to liver and bile, or via ingestion from mouth or nose secreta (saliva and mucus from the nose and bronchus). A qualitative model for the likelihood of shedding via faeces for the different administration routes used for replication-deficient HAdV-5 is shown in Figure 4. Since viral shedding seems to be both dose- and time-dependent, it is not possible to exclude shedding based on only a limited number of dose- or time-points. Published data, however, indicate that faecal shedding occurs in the first post-injection days, but not at later time-points. Thus, negative results from testing after the first week do not exclude the possibility of shedding at earlier time-points, whereas following negative testing in the first week, it may be concluded that also further shedding will be unlikely.

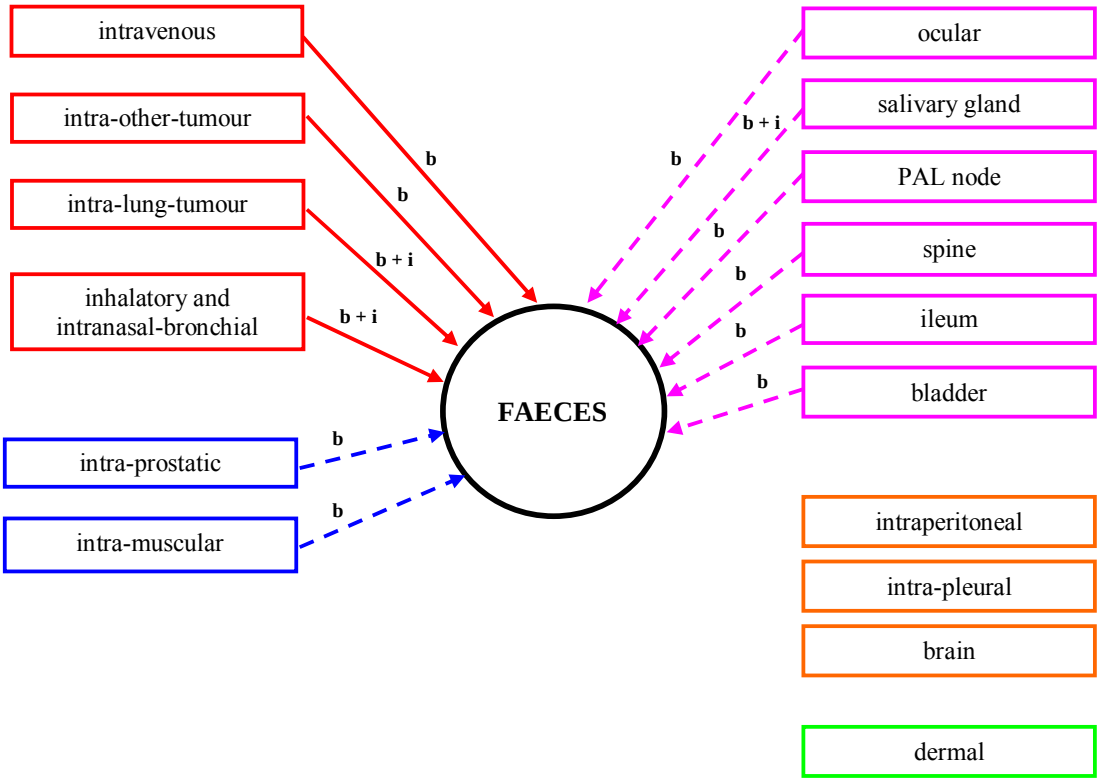

Figure 4. Qualitative model of HAdV-5 shedding via faeces from different administration routes. Arrow = shedding via faeces; dashed arrow = probable shedding via faeces; no arrow = no or unlikely shedding via faeces; b = via blood to liver and bile; i = via ingestion; red = tested and detected in faeces and blood; blue = tested and not detected in faeces, but detected in blood and/or other organs than site of administration; pink = not tested in faeces, but detected in blood and/or other organs than site of administration; orange = tested and not detected in faeces, blood or other organs than site of administration; green = not tested in faeces and not detected in blood and/or other organs than site of administration.

Shedding via faeces is a possible shedding route when virus is biodistributed to the liver, where after excretion through bile can result in positive stool samples. Indeed, several studies in mice, rabbits, pigs and monkeys have found biodistribution to the liver from 10 minutes up to 49 weeks after intravenous injection of HAdV-5 (39-45;47-52). Also positive shedding data following the intra-tumoral administration route are compatible with the data on biodistribution to the liver. After intra-tumoral administration to a lung tumour, also ingestion of mucus from the bronchus can be involved in shedding via faeces. Also after inhalatory and intranasal-bronchial administration, shedding was observed via faeces, probably due to ingestion of mucus, but it could also be caused by biodistribution to the blood and than via the liver and bile to the faeces.

Even though shedding via faeces was not detected 1 month after intra-prostatic injection (72), distribution to the liver was observed in dogs and mice (39;74). Therefore, the possibility of faecal shedding in the period directly after intra-prostatic injection can not be neglected. In addition, after administration to the salivary glands of rats shedding via faeces is possible and could be due to biodistribution to the liver (reported from 3 to 29 days) and due to ingestion of saliva (89;90). Animal studies show that HAdV-5 is also biodistributed to the liver following intra-muscular (51), intraperitoneal (39) and ocular administration (87), or after administration into the bladder (50). Therefore, also after these administration routes, shedding via faeces cannot be excluded.
Shedding via faeces seems unlikely following dermal, brain, intra-pleural and intraperitoneal administration, because no distribution to blood was observed and ingestion is not possible.

4.3.1.3 Shedding of HAdV-5 via mouth and nose secreta

A qualitative model for the likelihood of shedding via the mouth or nose secreta (saliva and mucus from nose and bronchus) for the different administration routes used for replication-deficient HAdV-5 is shown in Figure 5. There seem to be five possible routes involved in the shedding via the mouth or nose secreta: 1) via the blood to glands in mouth and nose and than to the mucus or saliva; 2) via mucus coughed up from the bronchus; 3) direct secretion of the viral vector into saliva or mucus; 4) direct attachment to saliva or mucus after administration (*e.g.* intranasal); or 5) secretion into the lachrymal fluid which goes to the nose and than to the mouth.
Shedding via nose and mouth secreta is very likely upon intra-tumoral administration of HAdV-5 for treatment of various lung cancers. Apparently, shedding can occur almost immediately after administration. In a range of tested dose levels, it was shown that the likelihood for shedding after intra-lung-tumoral administration is hardly influenced by the dose level (10^6 to 10^{11} pfu). In addition, also results upon administration in head and neck squamous cell carcinoma, suggest that shedding via nose and mouth secreta is occurring (68). From the fact that positive samples were detected upon administration of 10^{11} pfu HAdV-5 and no shedding was observed when lower dosages were administered, it can be hypothesised that shedding via nose and mouth secreta can be expected, although the levels of shedding will probably be lower compared to intra-tumorally injected lung cancer tumours. After inhalatory and intranasal-bronchial administration, the results indicate that mouth and/or nose shedding via saliva and mucus in nose or bronchus of HAdV-5 is likely, either via direct infection of the nose (intranasal administration), biodistribution to the blood and than to the secretion glands or via the mucus coughed up from the bronchus.
No shedding studies were performed after salivary gland administration, but it is likely that the viral vector will be directly secreted into the saliva. After intravenous, intra-prostatic, intra-muscular and ocular administration no shedding via the mouth or nose was observed. However, due to the biodistribution to the blood compartment shedding via mouth and/or nose secreta is possible. Also after ocular administration, it is possible that lachrymal fluid will be positive (not tested) and that via this route also the nose and mouth can become positive. In addition, after administration to spine, ileum or para-aortic lymph node in humans, blood samples were positive (54), indicating the possibility of further distribution and shedding via the mouth and/or nose. Since HAdV-5 can not pass the blood-brain barrier, leakage during administration most likely explains how HAdV-5 reaches the systemic circulation after injection into the spine.

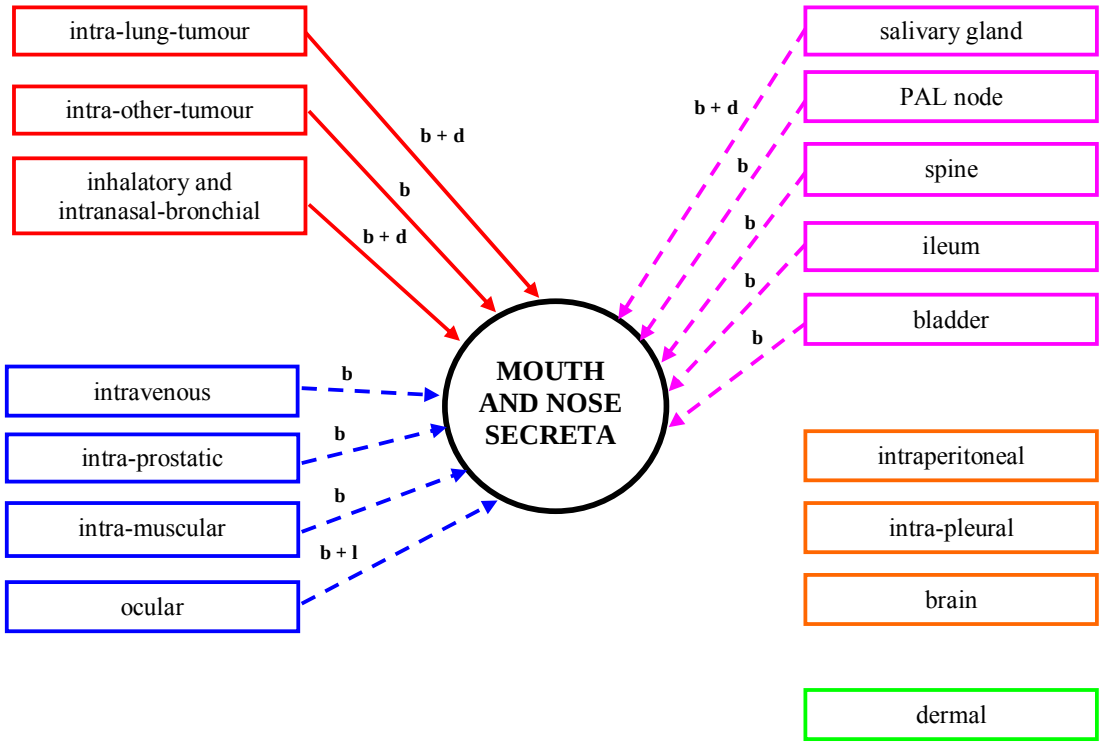

Figure 5. Qualitative model of HAdV-5 shedding via mouth and nose secreta from different administration routes. Arrow = shedding via mouth and/or nose secreta; dashed arrow = probable shedding via mouth and/or nose secreta; no arrow = no or unlikely shedding via mouth and/or nose secreta; b = via blood to mouth and/or nose secreta; d = direct to mouth or nose secreta; l = via lachrymal fluid to nose and mouth secreta; red = tested and detected in mouth and/or nose secreta and blood; blue = tested and not detected in mouth and/or nose secreta, but detected in blood and/or other organs than site of administration; pink = not tested in mouth and/or nose secreta, but detected in blood and/or other organs than site of administration; orange = tested and not detected in mouth and/or nose secreta, blood or other organs than site of administration; green = not tested in mouth and/or nose secreta and not detected in blood and/or other organs than site of administration.

It is unlikely that shedding via mouth or nose secreta will occur after intraperitoneal, intra-pleural and dermal administration and administration directly into the brain. Data indicate that after intraperitoneal, intra-pleural and dermal administration, HAdV-5 remains locally situated and does not biodistribute to the systemic circulation As discussed previously the absence of biodistribution upon HAdV-5 administration to the brain is due to the blood brain barrier which cannot be crossed by HAdV-5.

4.3.1.4 Shedding of HAdV-5 via semen

Shedding via semen is only possible if the viral vector is present in the seminal fluid or incorporated into or attached to the sperm cell. A qualitative model for the risk of shedding via semen for the different administration routes used for replication-deficient HAdV-5 is shown in Figure 6.

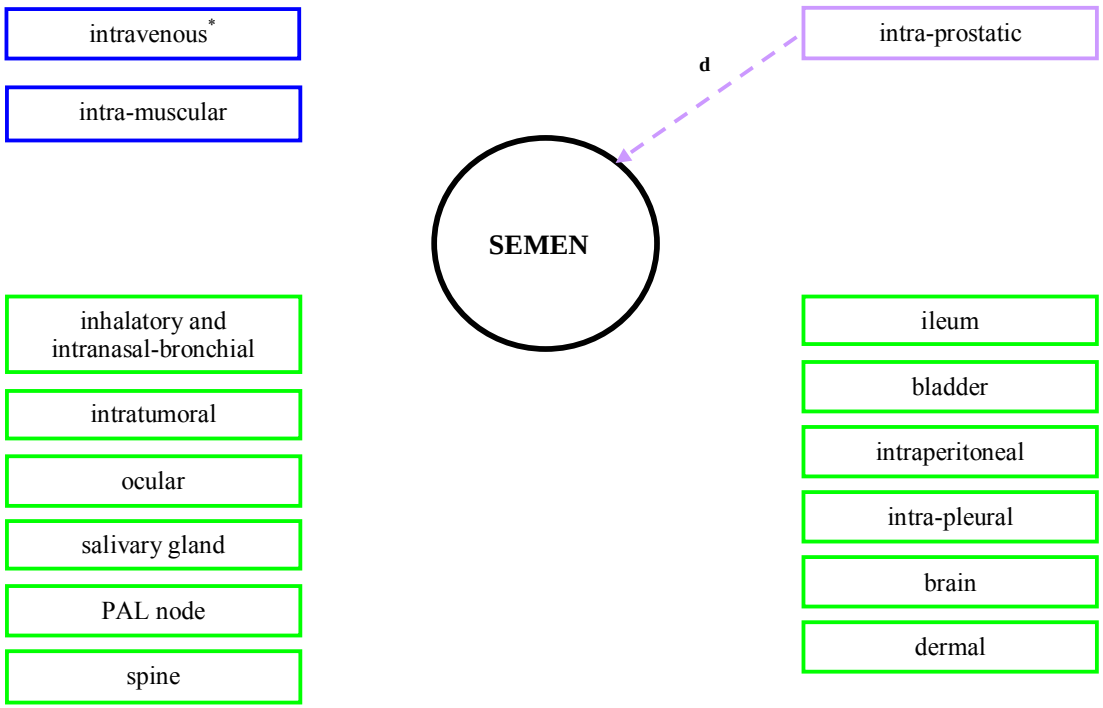

Figure 6. Qualitative model of HAdV-5 shedding via semen from different administration routes. Dashed arrow = probable shedding via semen; no arrow = no or unlikely shedding via semen; d = direct to semen; blue = tested and not detected in semen, but detected in blood and/or other organs than site of administration; purple = not tested in semen, but direct secretion into the seminal fluid is possible; green = not tested in semen and not detected in blood and/or other organs than site of administration; * semen was positive due to blood contamination of the samples.

The presence of HAdV-5 material was detected in sperm of rabbits two weeks following intravenous administration, but according to the authors this was most likely due to the presence of (positive) blood in the sample (44). Nevertheless, following intravenous administration distribution of HAdV-5 could also be found in the testes of rabbits and baboons (44;48), as well as in the prostate and ureter of mice (39), emphasizing the possibility of shedding into semen (50). After intra-muscular injection adenovirus was not detected in sperm 8 weeks following intra-muscular administration in humans (80), but other time-points were not analysed.

Unfortunately, shedding of HAdV-5 via semen is only marginally investigated. It can be speculated that shedding in semen can occur when viral particles are distributed to organs involved in the production of semen, such as the gonads and the organs involved in the excretion route of semen, such as the prostate and ureter. Therefore, especially intra-prostatic administration seems likely to result in shedding in semen. For the other administration routes described in this review, shedding via semen was not analysed at all, but unlikely because it seems that HAdV-5 present in the systemic circulation is not excreted via semen. Further studies are warranted to exclude shedding via semen as possibility.

4.3.1.5 Shedding of HAdV-5 via smaller excretion route

After dermal application, only local distribution was found and shedding was observed via the wound bed (71). Other HAdV-5 administration route studies did not investigate biodistribution to the skin or shedding via a wound bed. Therefore, it is likely that shedding via the wound is only relevant after dermal application. Nevertheless, the possibility of viral leakage through the needle shaft during or directly after administration via a needle (which obviously results in a small wound) should not be neglected.

Shedding via ear swabs was only studied by Herman *et al.* (1999) and did not occur within four days after intra-prostatic administration of 10^8 to 10^{11} iu (75). However, the detection method was based on culturing for wild type adenovirus. Although it is unlikely that shedding via ear fluid is a relevant route after administration of HAdV-5, it can not be excluded. In addition, shedding via the ear is probably of less risk for the environment of the patient than shedding via urine and faeces and via mouth and/or nose secreta.

Shedding via lachrymal fluid was not studied for HAdV-5, but it could be a relevant shedding route after ocular administration or biodistribution of the viral vector to the eye. Again, the risk for the environment of the patient will probably be much less than shedding via urine and faeces or via mouth and/or nose secreta.

4.3.1.6 Shedding of HAdV-5 via menstrual blood

Shedding through menstrual blood was not studied at all. However, this might be a more important route than via lachrymal fluid, ear, skin and wound exudate. It is therefore recommended to include menstrual blood in future clinical studies.

4.3.2 Adeno-associated 2 vector

The presence of heparan sulphate proteoglycans on the cell surface and the co-receptors c-Met and fibroblast growth factor receptor 1 directly correlate with the efficiency by which AAV can infect cells (33). c-Met is predominantly expressed in epithelial cells and in several non-epithelial cells, such as liver, neural, and skeletal muscle cells. In addition, fibroblast growth factor receptor 1 is relative abundant in skeletal muscle, neuroblasts and glioblasts in the brain (37). Following AAV2 administration to the bloodstream, spreading to the brain was detected, indicating that AAV2 is able to pass the blood brain barrier. This suggests that shedding could be possible following administration of such a viral vector to the brain.

4.3.2.1 Shedding of AAV2 via urine

A qualitative model for the likelihood of shedding via urine for the different administration routes used for AAV2 is shown in Figure 7. In general, there are three possible routes via which shedding via urine can occur; either via blood and kidney, directly from the bladder or from the prostrate to the ureter.

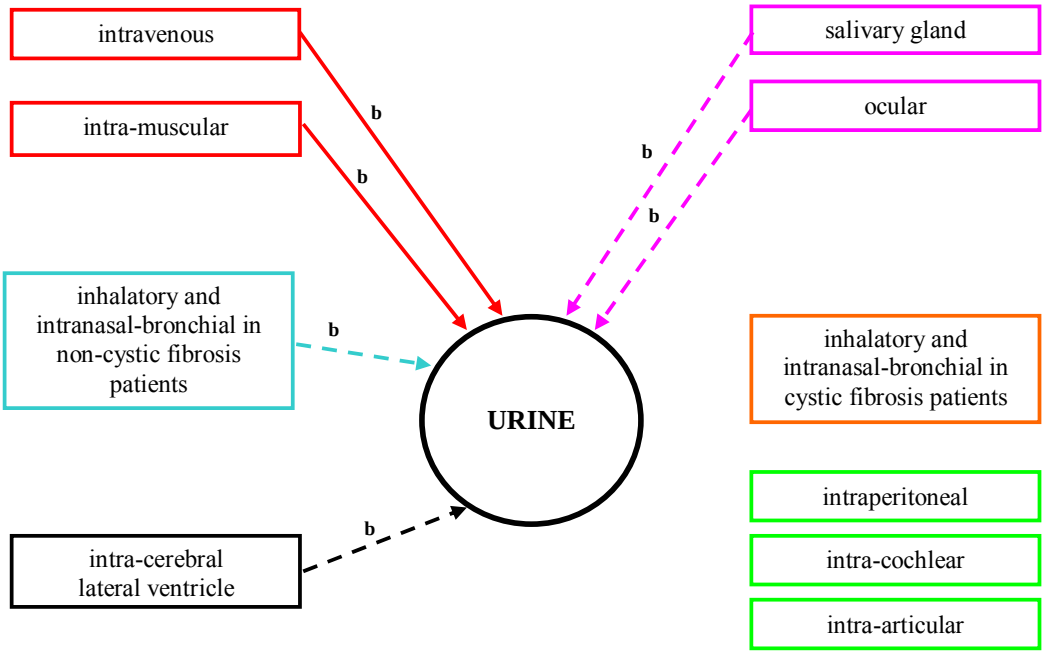

Figure 7. Qualitative model of AAV2 shedding via urine from different administration routes. Arrow = shedding via urine; dashed arrow = probable shedding via urine; no arrow = no or unlikely shedding via urine; b = via blood to urine; red = tested and detected in urine and blood; pink = not tested in urine, but detected in blood and/or other organs than site of administration; orange = tested and not detected in urine, blood or other organs than site of administration; aqua = not tested in non-cystic fibrosis patients, but could distribute to blood and other organs than site of administration due to different mucus features; green = not tested in urine and not detected in blood and/or other organs than site of administration; black = not tested outside the brain, but AAV2 can pass the blood-brain barrier.

After intravenous administration AAV2 could be detected in urine of monkeys for up to 6 days (100;101;109). Also in a clinical trial plasma samples remained positive for AAV2 sequences up to 12 weeks after administration (25). Also after intra-muscular administration, urine samples were positive up to 24 hours and negative thereafter.
Following delivery of AAV2 via aerosol nebulisation to the lungs of patients with cystic fibrosis, urine samples of all patients remained negative for shedding during the 90 days follow up (112). In agreement with these findings, also Wagner et al. (1999) did not detect AAV2 shedding in urine measured 2 days until 34 days after administration to the respiratory epithelium (114). In addition, Flotte et al. (2003) also did not detect AAV2 in urine samples collected between day 1 and 30 after intranasal and endo-bronchial administration (115). Although in these studies blood samples mostly tested negative for AAV2, one sample of thirty-one patients tested positive (112), thus shedding via urine is possible. It has been reported that both physical and biological barriers in the airways might cause an inhibitory effect on viral transduction and biodistribution (144). The airway surface fluid and

mucous layers are natural barriers in the airway that protect against bacterial and viral infections and the intense inflammatory milieu of the cystic fibrosis airway associated with hyper secretion of mucous may act as an increased barrier. Thick inflammatory sputum from cystic fibrosis patients has been demonstrated to block and/or inactivate AAV-mediated transduction of cells *in vitro* (145). Additionally, preferential transduction from the basolateral membrane of polarised airway epithelia has also been observed for AAV (146). It could be speculated that these barriers prevent efficient biodistribution of AAV2 upon airway administration. However, it should be noted that these barriers will not display a 100% inhibition. Therefore, it is not surprising that in some cases AAV2 blood positive samples were detected. In non-cystic fibrosis patients, the physical barrier is less and it could be expected that in non-cystic fibrosis patients the biodistribution of AAV2 after an airway transmission would occur with increased efficiency. Thus, excretion of AAV2 via urine can not be excluded in non-cystic fibrosis patients, but is unlikely in cystic fibrosis patients.
Studies that applied other administration routes (salivary gland, ocular, intra-cerebral lateral ventricle, intraperitoneal, intra-cochlear and intra-articular) did not investigate the excretion of AAV2 in urine. Some of these studies did test the presence of AAV2 in a variety of other tissues. Particularly when AAV2 is detected in blood, kidney or bladder, one should consider the possibility that AAV2 will be excreted via urine.

4.3.2.2 Shedding of AAV2 via faeces

Shedding via faeces can occur via two possible routes; either via blood to liver and bile or via ingestion from mouth or nose secreta (saliva and mucus from the nose and bronchus). A qualitative model for the likelihood of shedding via faeces for the different administration routes used for AAV2 is shown in Figure 8.
Faeces samples were positive in monkeys after administration to muscles in the legs (109). In human, no shedding was detected in faeces after injection into the *in vastus lateralis*, but serum was positive (111). The difference between monkey and human could be due to variations in analysis method used to detect AAV2 in faeces, but could also be due to differences in muscle injected. In addition, species differences could also account for the opposite results.
After inhalatory and intranasal-bronchial administration to cystic fibrosis patients, stool samples were negative from 1 to 90 days (112;114;115). Also blood was mostly negative after inhalatory or intranasal-bronchial administration (only one sample in 1 out of 31 patients). As explained for shedding via urine for AAV2, the thick inflammatory sputum and changes in epithelial cells in cystic fibrosis patients could result in reduced biodistribution of the viral vector to the systemic circulation after inhalatory and intranasal-bronchial administration (145). However, saliva was positive and HAdV-5 could thus be shed via faeces due to ingestion of the saliva. In non-cystic fibrosis patients, the physical barrier is less and therefore, in non-cystic fibrosis patients a wider biodistribution pattern of AAV2 and excretion of AAV2 via faeces can not be excluded.
Other studies did not investigate shedding via faeces, but in some studies biodistribution to blood or other organs, including the liver was investigated. If biodistribution to blood or liver is observed, than shedding via faeces of AAV2 could be possible.

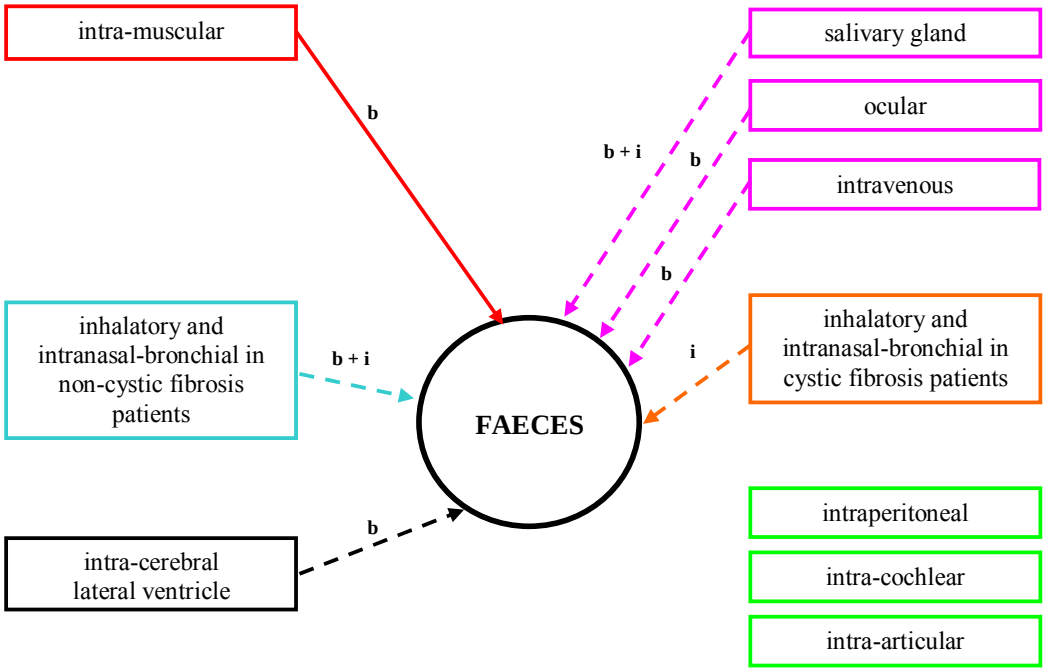

Figure 8. Qualitative model of AAV2 shedding via faeces from different administration routes. Arrow = shedding via faeces; dashed arrow = probable shedding via faeces; no arrow = no or unlikely shedding via faeces; b = via blood to liver and bile; i = via ingestion; red = tested and detected in faeces and blood; pink = not tested in faeces, but detected in blood and/or other organs than site of administration; orange = tested and not detected in faeces, blood or other organs than site of administration; aqua = not tested in non-cystic fibrosis patients, but could distribute to blood and other organs than site of administration due to different mucus features; green = not tested in faeces and not detected in blood and/or other organs than site of administration; black = not tested outside the brain, but AAV2 can pass the blood-brain barrier.

4.3.2.3 Shedding of AAV2 via mouth and nose secreta

A qualitative model for the likelihood of shedding via mouth or nose secreta (saliva and mucus from nose and bronchus) for the different administration routes used for AAV2 is shown in Figure 9. There seem to be four possible routes involved in the shedding via mouth or nose secreta: 1) via the blood to glands in mouth and nose and than to the mucus or saliva; 2) via mucus coughed up from the bronchus; 3) direct secretion of the viral vector into saliva or mucus; or 4) secretion into the lachrymal fluid which goes to the nose and than to the mouth.

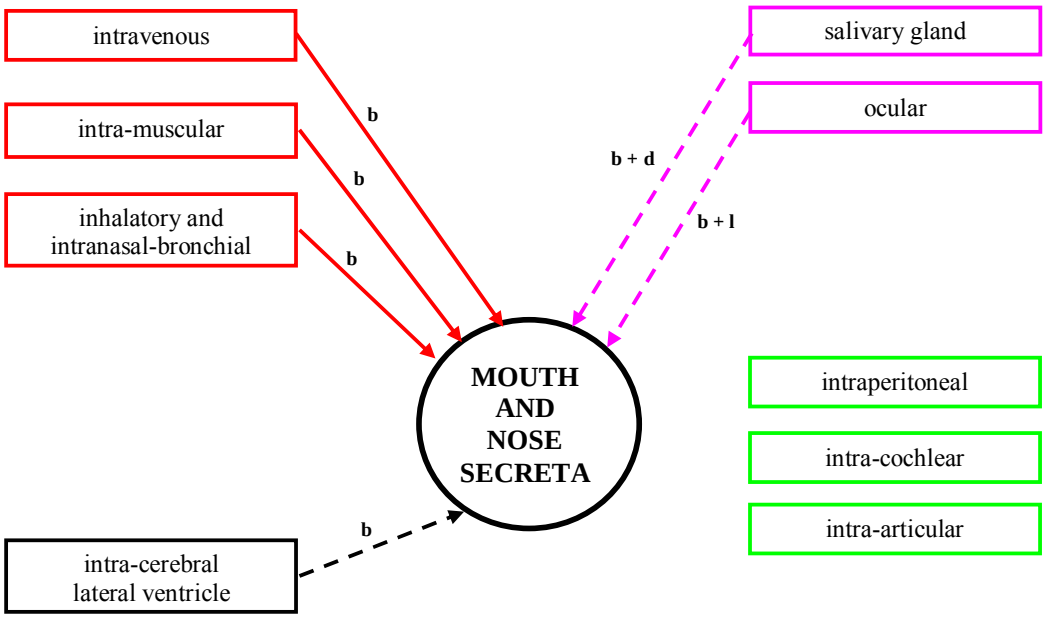

Figure 9. Qualitative model of AAV2 shedding via mouth and nose from different administration routes. Arrow = shedding via mouth and/or nose secreta; dashed arrow = probable shedding via mouth and/or nose secreta; no arrow = no or unlikely shedding via mouth and/or nose secreta; b = via blood to mouth and/or nose secreta; d = direct to mouth or nose secreta; l = via lachrymal fluid to nose and mouth secreta; red = tested and detected in mouth and/or nose secreta and blood; pink = not tested in mouth and/or nose secreta, but detected in blood and/or other organs than site of administration; green = not tested in mouth and/or nose secreta and not detected in blood and/or other organs than site of administration; black = not tested outside the brain, but AAV2 can pass the blood-brain barrier.

Saliva of monkeys tested positive for viral DNA after administration of AAV2 to the hepatic artery (100). Other studies after intravenous administration did not investigate shedding via saliva or mucus. In human haemophilia patients, saliva was also positive for viral DNA up to 2 weeks after intra-muscular administration to leg muscles, but negative afterwards (25). Saliva was also positive for the first 24 hours after *in vastus lateralis* administration in human haemophilia patients and was negative at 48 hours (111). In addition, Rhesus and Cynomolgus monkeys treated in leg muscles showed shedding via saliva for the first 2 days (109). Also after inhalatory administration to human cystic fibrosis patients, sputum was positive for 150 days in an infectious assay (113). However, in two other studies different shedding results were obtained in human cystic fibrosis patients. In one study patients were treated intranasal-bronchial and sputum was positive on day 1 for viral DNA and negative on day 7 to 30 (115). In the other study, sputum was positive on day 1 and 7 and negative on day 14 and 30 (112). Differences in analysis techniques used could explain the differences in results. Nevertheless, it is obvious that shedding via mouth or nose secreta occurs after administration to the respiratory system, although for the exact duration of shedding more data are needed.
Data on shedding via mouth and nose secreta after other administration routes are not available. The data indicate that saliva and nose mucus are possible routes for shedding if the viral vector reaches the systemic circulation after administration or when shedding via lachrymal fluid is possible. However, shedding occurs for a certain period of time until blood levels are too low to generate sufficient biodistribution to mouth and nose secreta.

4.3.2.4 Shedding of AAV2 via semen

Shedding via semen is only possible if the viral vector is present in the seminal fluid or incorporated into or attached to the sperm cell. A qualitative model for the risk of shedding via semen for the different administration routes used for AAV2 is shown in Figure 10.

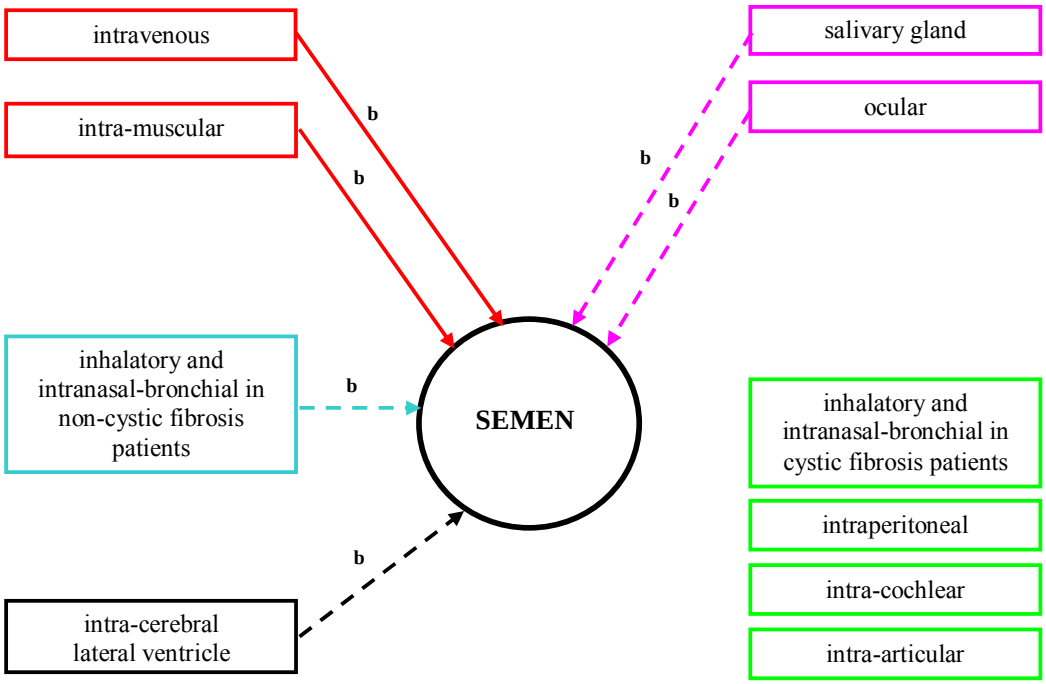

Figure 10. Qualitative model of AAV2 shedding via semen from different administration routes. Arrow = shedding via semen; dashed arrow = probable shedding via semen; no arrow = no or unlikely shedding via semen; b = via blood to gonads and semen; red = tested and detected in semen and blood; pink = not tested in semen, but detected in blood and/or other organs than site of administration; aqua = not tested in non-cystic fibrosis patients, but could distribute to blood and other organs than site of administration due to different mucus features; green = not tested in semen and not detected in blood and/or other organs than site of administration; black = not tested outside the brain, but AAV2 can pass the blood-brain barrier.

Shedding of AAV2 via semen or epididymal effluent has been reported after intravenous (92;93;95) and intra-muscular (93;103;104) administration to rat, rabbit, and human, but not in dog. Gonads were also tested positive for AAV2 in mice, rats and rabbits (93;95). In a study with adult men having haemophilia B, AAV2 delivered to the liver via the hepatic artery resulted in transient vector dissemination to the semen and it was concluded that clearance from semen is not dose dependent (92). In addition, it was observed that the clearance from semen depends on the age of the treated individuals.

Submandibular administration (117) also resulted in biodistribution of AAV2 to the testis. Unfortunately, there are no data on shedding via semen, but the possibility of shedding via semen can not be excluded.

After administration of AAV2 via intraperitoneal injection, the retina (116) and isolated limb perfusion (107), testis tested negative for AAV2. For other administration routes biodistribution to gonads or

shedding via semen has not been investigated. When shedding via semen is expected based on the presence of viral particles in the circulation, the risk of shedding via semen will be reduced when a waiting period (2 to 3 spermatogenesis periods, thus between 130-210 days in humans) is taken into account after blood, plasma or serum becomes negative for viral particles.

4.3.2.5 Shedding of AAV2 via smaller excretion routes

A lachrymal swab from Rhesus and Cynomolgus monkeys was positive for 6 hours to 6 days after intra-muscular administration (109) and then returned to negative. These results indicate that shedding via this route could be relevant. No other studies investigated shedding via lachrymal fluid. After ocular administration, the eye was positive in dog and monkey (99;116) for periods of 3 months and 1 week, respectively. Thus shedding via lachrymal fluid can be expected following ocular administration. No other biodistribution studies were performed investigating the biodistribution to the eye.
After intra-cochlear administration, both the injected and control cochlea were positive during the sampling period of 4 weeks (120). However, shedding via the ear was not investigated in this study or in studies that used other administration routes. It could be a shedding route, but probably of less risk for the patient's environment than the other shedding routes.
The skin of monkeys was positive at 3 month after intravenous administration (122). However, this was not confirmed in other monkey studies (122). If the skin is positive, than a possible shedding route would be the loss of the stratum corneum, but this probably does not result in a risk for the environment. Shedding via a wound bed is most likely only relevant if the wound bleeds or lymph is excreted, which could occur in most administration routes during or directly after viral administration via the needle puncture.

4.3.2.6 Shedding of AAV2 via menstrual blood

Shedding through menstrual blood was not studied at all. However, this might be a more important shedding route than via lachrymal fluid, ear, skin or wound exudate. It is therefore recommended to include menstrual blood in future clinical studies.

5 Conclusions and recommendations

5.1 Conclusions

Gene therapy is a rapidly developing field to treat diseases (*e.g.* haemophilia, cystic fibrosis, cancer). Although gene therapy testing in humans has advanced rapidly, many questions concerning its use are still left. Regarding safety and risk assessment of viral vectors used in gene therapy, biodistribution and shedding play a pivotal role in predicting the risk for the environment of the patient. This report presents an overview on biodistribution and shedding data of HAdV-5 and AAV2 related to the used administration route. The conclusions in this report are only valid for replication-deficient HAdV-5 and AAV2 and can not be extrapolated to other serotypes and other viral vectors.

Biodistribution of gene therapy viral vectors is mostly investigated in animal models. However, in this report the focus is on HAdV-5 and AAV2, which both originate from wild type viruses with a human host. Differences between species in receptors needed for viral infection (12;13;17-19), erythrocyte binding (20) or the presence of neutralising antibodies (139;140) can influence the biodistribution profile. To extrapolate the spreading of an adenoviral vector from an animal model to humans, it is important to be aware of these differences. Thus, data from animal models, especially rodents, have a relatively poor predictive power for humans.

HAdV-5 data from intravenous and brain administration indicate that HAdV-5 viral vector is not transported from the bloodstream to the brain or vice versa. As a result, shedding does not occur after delivery of recombinant HAdV-5 to the brain. This is in agreement with reports that identify the blood-brain barrier as a main obstacle for intravascular administered HAdV-5 vectors to enter brain cells (140;147;148). In contrast, HAdV-5 was detected by PCR in the plasma of patients to which HAdV-5 was injected directly in the brain following tumour resection. The authors explained this observation by indicating that some systemic release of the adenovirus can occur during surgery, presumably via blood vessels exposed during the tumour resection (149). In general, transport of HAdV-5 across the blood-brain barrier in humans is not expected at the current dosages used in gene therapy. In contrast to HAdV-5, AAV2 could be detected in the brain after administration to the blood stream (101;150). This is in agreement with previous reports indicating that AAV can be shuttled over the blood-brain barrier by transcytosis (79).

A general qualitative model for the shedding risk of HAdV-5 and AAV2 via the different excretion routes is shown in Figure 11 and Figure 12, respectively. One has to keep in mind that the shedding that is described in these figures includes both (but does not distinguish between) the shedding of infectious viral particles and the shedding of non-infectious particles and viral vector DNA. After intravenous administration and after administration via routes that lead to biodistribution to the systemic circulation, shedding via urine, faeces, mouth and nose secreta, lachrymal fluid and menstrual blood is expected for both HAdV-5 and AAV2. However, the more compartments the viral vector has to pass to reach a certain excretion route, the less the risk of shedding will be due to either dilution of the viral vector, degradation in case of passage through the stomach or interaction with the immune system. In addition, shedding via semen is also expected for AAV2, but not for HAdV-5. In cystic fibrosis patients, inhalatory and intranasal-bronchial administration resulted in local distribution without biodistribution to the systemic circulation, but still shedding via mouth and nose secreta and also faeces is possible. However, in non-cystic fibrosis patients, biodistribution to the systemic circulation can be expected due to differences in the barrier formed by the mucus and epithelial cell layer of the inhalatory tract. Therefore, also shedding via other routes can be expected in non-cystic fibrosis patients.

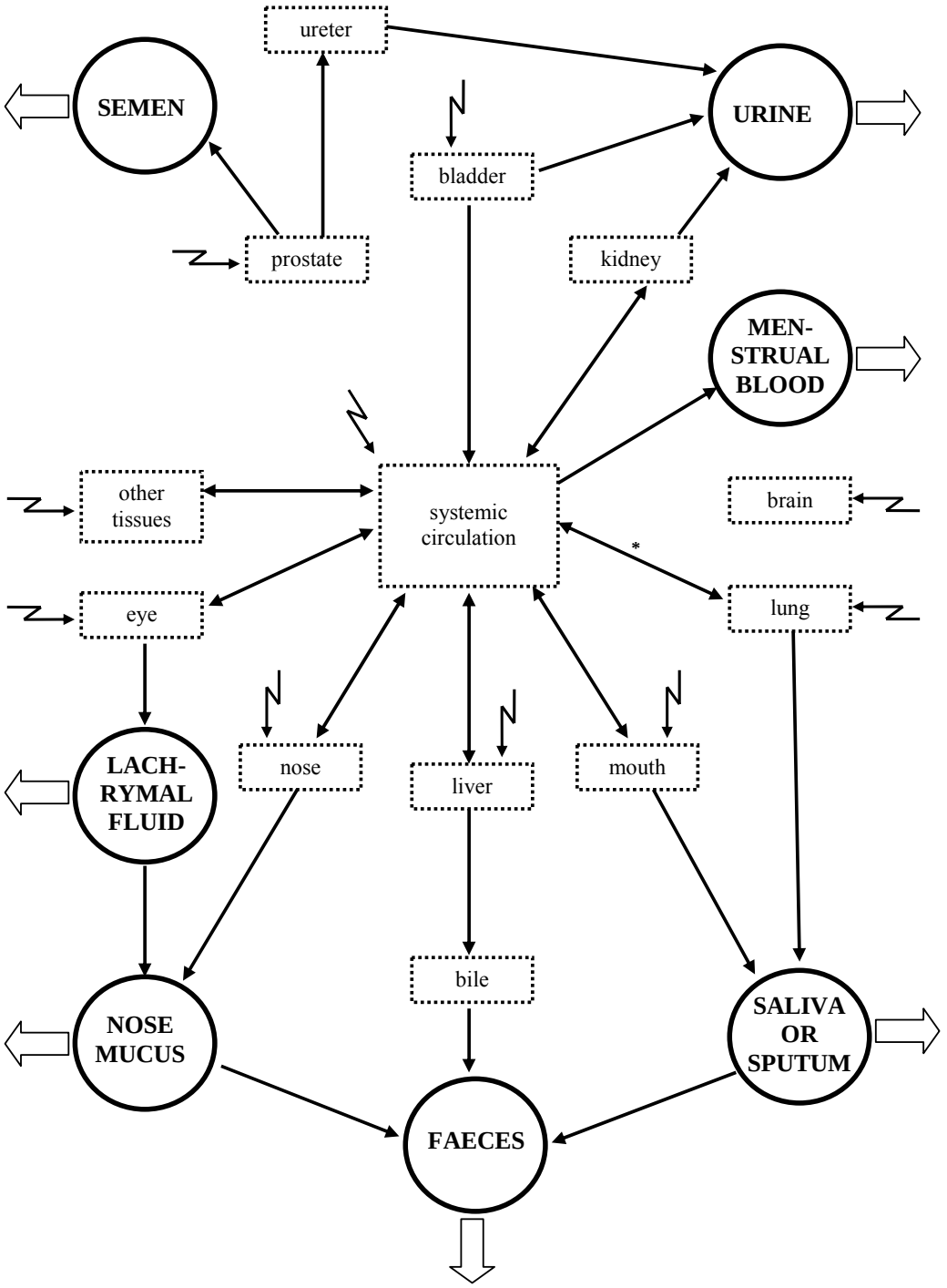

Figure 11. Overall qualitative model for the shedding risk of HAdV-5. ⇝ = route of administration; ⇨ = shedding of viral vector; ⟶ = biodistribution of viral vector; * = unlikely in cystic fibrosis patients; ⬚ = organs and tissues; ◯ = excreta.

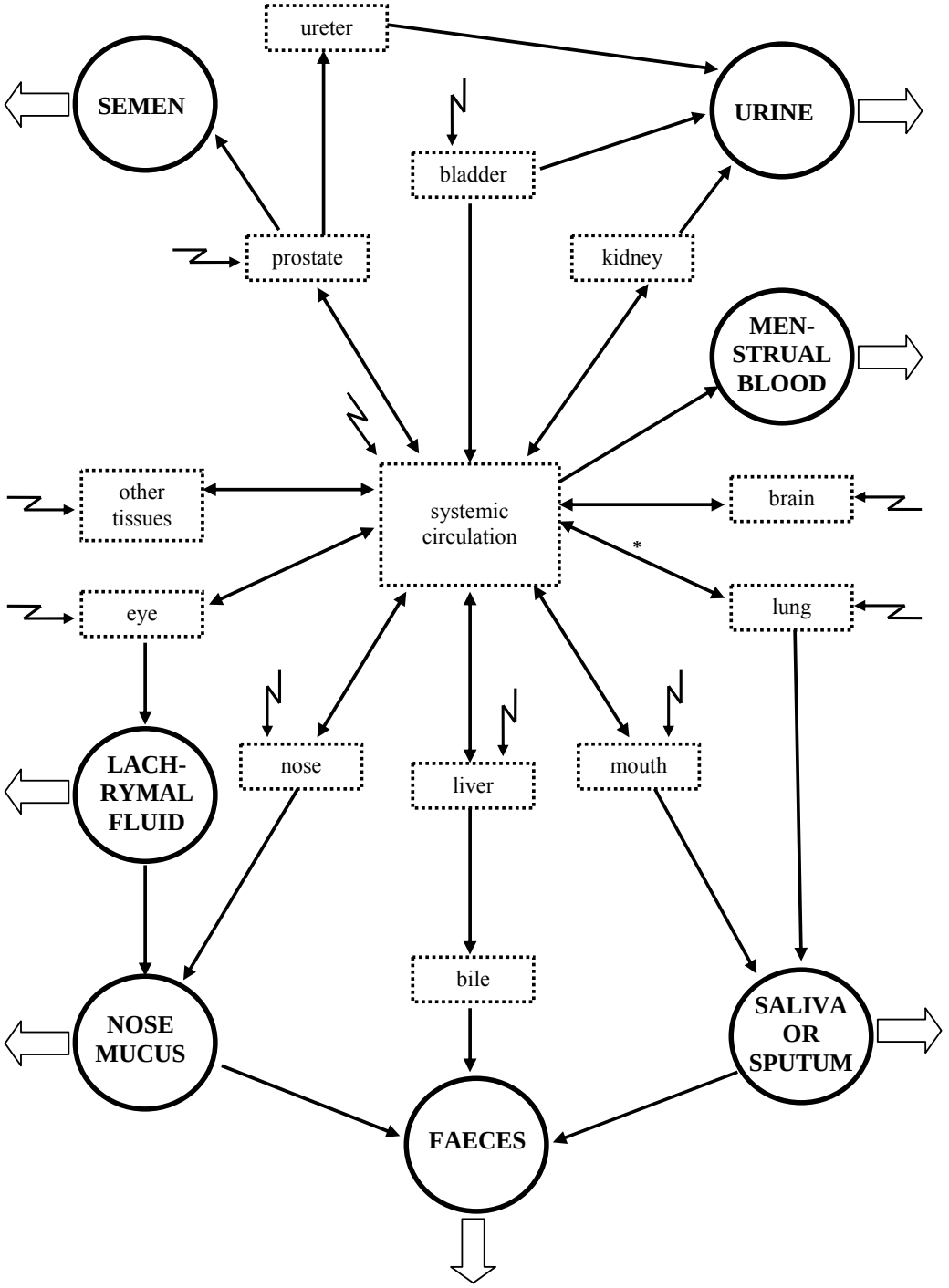

Figure 12. Overall qualitative model for the shedding risk of AAV2. ⇝ = route of administration; ⇨ = shedding of viral vector; ⟶ = biodistribution of viral vector; * = unlikely in cystic fibrosis patients; ▭ = organs and tissues; ◯ = excreta.

In conclusion, the routes of shedding for both viral vectors depend on the route of administration. Some routes lead to local biodistribution (*e.g.* intraperitoneal) and thus to no shedding or to local shedding only. Other routes lead to systemic biodistribution (*e.g.* intra-muscular)and to shedding via several excretion routes. In fact, in any case when systemic biodistribution is observed, shedding via all excreta, relevant for that viral vector, might occur. Shedding via semen is expected for AAV2, but not for HAdV-5. Transport across the blood-brain barrier can be expected for AAV2, but not for HAdV-5, except when the blood-brain barrier is damaged. These models can help researchers and risk assessors in predicting the different shedding routes after a certain administration route. The conclusions in this report are valid only for replication-deficient HAdV-5 and AAV2. Especially negative shedding results for replication-deficient HAdV-5 and AAV2 do not automatically indicate that shedding via that excretion route is also not possible for replication-competent HAdV-5 and AAV2, for instance due to increase in viral vector amount. However, the positive shedding results can be extrapolated to replication-competent HAdV-5 and AAV2 viral vectors, although they can not be extrapolated to other serotypes or viral vectors.

5.2 Recommendations

Good (pre-)clinical kinetic studies are warranted, in which biodistribution and shedding are investigated in the relevant tissues and excreta routes and for a relevant time frame. However, investigators should be aware of existing species differences. Kinetic studies in non-naïve monkey and pig are preffered for kinetic studies using viral vectors and studies in rodents are the least relevant due to the major interspecies differences. In addition, more experimental details should be provided in the literature for data interpretation and to facilitate the comparison of studies. Also, researchers should measure shedding with infectious assays and not PCR, because only infectious assays provide information on the shedding of infectious viral particles, which have entirely different risks in the environment than non-infectious particles or DNA, that are measured with PCR.
Secondly, the excretion routes lachrymal fluid and menstrual blood should be studied more extensively, because they can be a shedding route for both HAdV-5 and AAV2. Thus, could pose a possible risk for the patient's environment. Also other minor excretion routes like wound exudate, skin and sweat should be investigated to rule out possible shedding via these routes.
The qualitative models provided in this review can help to setup the most relevant pre-clinical *in vivo* experiments and clinical trials. In addition, a better setup (*e.g.* sampling time and tissues sampled) of the kinetic studies in gene therapy clinical trials could help to interpret shedding data and indicate which routes are the most important. Furthermore, these additional data could help to make a quantitative model, in order to predict shedding based on the type of viral vector used in combination with its administration route. The proposed qualitative models can help risk assessors to determine the potential risk of shedding via the different excretion routes. Finally, it can help regulators in setting up guidance, which describes how clinical trials have to be set-up (*e.g.* samples taken, time frame) and which non-clinical studies should be performed before starting clinical trials. Overall, these models are recommended to be used in setting-up research for and risk assessment of new HAdV-5 and AAV2 viral vectors used in gene therapy.

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Appendix A. Biodistribution and shedding of HAdV-5 and AAV2 per administration route

In the following appendix the biodistribution and shedding is shown per administration route for HAdV-5 (Table A.1 to A.15) and AAV2 (Table A.16 to A.24).

Table A.1. Intravenous and intra-arterial administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
human	$3*10^{10}$ - $3*10^{12}$ vp	oral rinse	1-28 days	-	(46)
		plasma	1 day	+	(46)
			2 days	+	(46)
			5 days	+	(46)
			14 days	+	(46)
			28 days	+	(46)
		rectal swab	1-28 days	+	(46)
		urine	1-28 days	+	(46)
human	$3*10^{10}$ vp	plasma	1 day	+	(46)
			2 days	-	(46)
			5 days	-	(46)
			14 days	-	(46)
			28 days	-	(46)
mice	$5*10^{10}$ vp	kidney	90 minutes	+	(49)
		liver	90 minutes	+	(49)
		lung	90 minutes	+	(49)
human	$1*10^{11}$ vp	plasma	1 day	+	(46)
			2 days	+	(46)
			5 days	-	(46)
			14 days	-	(46)
			28 days	-	(46)
mice	$1*10^{11}$ vp	blood cells	10 minutes	+	(41)
		liver	10 minutes	+	(41)

		lung	10 minutes	+	(41)
		serum	10 minutes	+	(41)
		spleen	10 minutes	+	(41)
human	$3*10^{11}$ vp	plasma	1 day	+	(46)
			2 days	-	(46)
			5 days	+	(46)
			14 days	+	(46)
rabbit	$9.5*10^{11}$ vp	blood	5 minutes	+	(43)
			30 minutes	+	(43)
			2 hours	+	(43)
			1 day	+	(43)
		eye	1 day	+	(43)
		heart	1 day	+	(43)
		kidney	1 day	+	(43)
		liver	1 day	+	(43)
		lung	1 day	+	(43)
		ovary	1 day	+	(43)
		spleen	1 day	+	(43)

human	$1*10^{12}$ vp	plasma	1 day	+	(46)
			2 day	+	(46)
			5 days	+	(46)
			14 days	+	(46)
			28 days	+	(46)
		tumour biopsy	4 days	+	(46)
pig	$1*10^{12}$ vp	liver	24 hours	+	(51)
		lung	24 hours	+	(51)
		spleen	24 hours	+	(51)
		ventricle, left	24 hours	-	(51)
monkey (Baboon)	$2*10^{12}$ vp/kg	blood leukocytes, peripheral	72 hours	-	(48)
		bone marrow MNC	72 hours	+	(48)
		brain	72 hours	+	(48)
		heart	72 hours	+	(48)
		duodenum	72 hours	+	(48)
		esophagus	72 hours	+	(48)
		kidney	72 hours	+	(48)
		liver	72 hours	+	(48)
		lung	72 hours	+	(48)

		lymph nodes, axillary	72 hours	+	(48)
		lymph nodes, inguinal	72 hours	+	(48)
		lymph nodes, mesenteric	72 hours	+	(48)
		pancreas	72 hours	+	(48)
		spleen	72 hours	+	(48)
		stomach	72 hours	+	(48)
		testis	72 hours	+	(48)
human	$3*10^{12}$ vp	tumour biopsy	4 days	+	(46)
mice	$1*10^{8}$ pfu	heart	1 week	+	(42)
		kidney	1 week	+	(42)
			2 weeks	+	(39)
		liver	1 week	+	(42)
			2 weeks	+	(39)
		lung	1 week	+	(42)
			2 weeks	+	(39)
		prostate	2 weeks	+	(39)
		spleen	1 week	+	(42)
			2 weeks	+	(39)
mice	$1*10^{9}$ pfu	bile	10 minutes	+	(40)
	(=$1.2*10^{11}$ vp)		90 minutes	+	(40)
			8 hours	+	(40)
			24 hours	+	(40)

		liver	10 minutes	+	(40)
			30 minutes	+	(40)
			90 minutes	+	(40)
			3 hours	+	(40)
			8 hours	+	(40)
			24 hours	+	(40)
			1 week	+	(40)
			2 weeks	+	(40)
			6 weeks	+	(40)
mice	$5*10^9$ pfu	bladder	72 hours	+	(50)
		heart	72 hours	+	(50)
		kidney	72 hours	+	(50)
		liver	72 hours	+	(50)
		lung	72 hours	+	(50)
		ureter	72 hours	+	(50)
rabbit	$1.15*10^{10}$ pfu	aorta	2 weeks	+	(52)
		blood cells, white	2 weeks	+	(44)
		blood vessel, control	2 weeks	-	(44)
		blood vessel, target	2 weeks	+	(44)
		bone marrow	2 weeks	+	(44)
		cerebellum	2 weeks	-	(44)

		cerebra	2 weeks	-	(44)
		epididymis	2 weeks	-	(44)
		heart	2 weeks	+/- #	(44)
		kidney	2 weeks	+/- #	(44)
		liver	2 weeks	+	(52)
			2 weeks	+	(44)
		lung	2 weeks	+	(44)
		lymph node	2 weeks	+	(44)
		muscle, skeletal	2 weeks	+	(44)
		sperm	2 weeks	+	(44)
		spleen	2 weeks	+/- #	(44)
		testis	2 weeks	+	(44)
mice	$3*10^9$ iu	kidney	2 weeks	+	(47)
			28 weeks	+	(47)
			49 weeks	+	(47)
		liver	2 weeks	+	(47)
			28 weeks	+	(47)
			49 weeks	+	(47)
		lung	2 weeks	+	(47)
			28 weeks	+	(47)
			49 weeks	+	(47)

		spleen	2 weeks	+	(47)
			28 weeks	+	(47)
			49 weeks	+	(47)
mice	$1*10^{10}$ iu	blood	1 minute	+	(53)
			5 minutes	+	(53)
			10 minutes	+	(53)
			20 minutes	+	(53)
			30 minutes	+	(53)
			60 minutes	+	(53)
rabbit	$5*10^{10}$ iu	plasma	10 minutes	+	(45)
			30 minutes	+	(45)
			60 minutes	+	(45)
			4 hours	+	(45)
			24 hours	+	(45)
			48 hours	+	(45)
rabbit	$5*10^{11}$ iu	plasma	10 minutes	+	(45)
			30 minutes	+	(45)
			60 minutes	+	(45)
			4 hours	+	(45)
			24 hours	+	(45)
			48 hours	+	(45)
		liver	60 days	+	(45)

vp = vector particles
pfu = plaque forming units
iu = infectious units
[#] = depending on infused artery
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.2. Intraperitoneal and intra-pleural administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
human	$3*10^{10}$ vp	ascites	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
		peritoneal washings	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
		sputum	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
		stool	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
		urine	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
human	$7.5*10^{10}$ vp	biopsy, laparoscopic	6-7 days	+	(56)
			> 1 year	+	(56)
		fluid, ascitic	6-7 days	+	(56)
			> 1 year	+	(56)

		fluid, pleural	72 h	+	(56)
		fluid, peritoneal	6-7 days	+	(56)
			> 1 year	+	(56)
		stool	1-28 days	-	(56)
		urine	1-28 days	-	(56)
human	$3*10^{11}$ vp	ascites	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
		peritoneal washings	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
		sputum	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
		stool	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
		urine	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)

human	$7.5*10^{11}$ vp	biopsy, laparoscopic	6-7 days	+	(56)
			> 1 year	+	(56)
		fluid, ascitic	6-7 days	+	(56)
			> 1 year	+	(56)
		fluid, pleural	72 h	+	(56)
		fluid, peritoneal	6-7 days	+	(56)
			> 1 year	+	(56)
		stool	1-28 days	-	(56)
		urine	1-28 days	-	(56)
human	$1*10^{12}$ vp	ascites	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
		peritoneal washings	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
		sputum	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
		stool	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)

		urine	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
human	$2.5*10^{12}$ vp	biopsy, laparoscopic	6-7 days	+	(56)
			> 1 year	+	(56)
		fluid, ascitic	6-7 days	+	(56)
			> 1 year	+	(56)
		fluid, pleural	72 h	+	(56)
		fluid, peritoneal	6-7 days	+	(56)
			> 1 year	+	(56)
		stool	1-28 days	-	(56)
		urine	1-28 days	-	(56)
human	$3*10^{12}$ vp	ascites	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
		peritoneal washings	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
		sputum	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)

		stool	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
		urine	1-6 days	-	(55)
			8 days	-	(55)
			15 days	-	(55)
			22 days	-	(55)
human	$7.5*10^{12}$ vp	biopsy, laparoscopic	6-7 days	+	(56)
			> 1 year	+	(56)
		fluid, ascitic	6-7 days	+	(56)
			> 1 year	+	(56)
		fluid, pleural	72 h	+	(56)
		fluid, peritoneal	6-7 days	+	(56)
			> 1 year	+	(56)
		stool	1-28 days	-	(56)
		urine	1-28 days	-	(56)
human	$1.5*10^{13}$ vp	blood	1 day	-	(57)
			3 days	-	(57)
			5 days	-	(57)
			7 days	-	(57)
			14 days	-	(57)
			19 days	-	(57)
		nasal swab	1 day	-	(57)
			3 days	-	(57)

			5 days	-	(57)
			7 days	-	(57)
			14 days	-	(57)
			19 days	-	(57)
		urethral swab	1 day	-	(57)
			3 days	-	(57)
			5 days	-	(57)
			7 days	-	(57)
			14 days	-	(57)
			19 days	-	(57)
		rectal swab	1 day	-	(57)
			3 days	-	(57)
			5 days	-	(57)
			7 days	-	(57)
			14 days	-	(57)
			19 days	-	(57)
human	$2.5*10^{13}$ vp	biopsy, laparoscopic	6-7 days	+	(56)
			> 1 year	+	(56)
		fluid, ascitic	6-7 days	+	(56)
			> 1 year	+	(56)
		fluid, pleural	72 h	+	(56)
		fluid, peritoneal	6-7 days	+	(56)
			> 1 year	+	(56)
		stool	1-28 days	-	(56)
		urine	1-28 days	-	(56)

human	$7.5*10^{13}$ vp	biopsy, laparoscopic	6-7 days	+	(56)
			> 1 year	+	(56)
		fluid, ascitic	6-7 days	+	(56)
			> 1 year	+	(56)
		fluid, pleural	72 h	+	(56)
		fluid, peritoneal	6-7 days	+	(56)
			> 1 year	+	(56)
		stool	1-28 days	-	(56)
		urine	1-28 days	-	(56)
mice	$1*10^{8}$ pfu	kidney	14 days	+	(39)
		liver	14 days	+	(39)
		lung	14 days	-	(39)
		prostate	14 days	+	(39)
		spleen	14 days	+	(39)

vp = vector particles
pfu = plaque forming units
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.3. Intra-tumoural administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
human	$1*10^{8}$-$5*10^{11}$ vp	blood	30 minutes	+	(63)
			1 hour	+	(63)
			2 hours	+	(63)
			4 hours	+	(63)
			8 hours	+	(63)
			24 hours	+	(63)
		plasma	24 hours	-	(63)
		stool	24 hours	-	(63)
		throat swab	24 hours	-	(63)
		urine	24 hours	-	(63)
human	$4*10^{9}$-$4*10^{11}$ vp	blood	2 weeks	-	(67)
		sputum	2 weeks	-	(67)
		urine	2 weeks	-	(67)
human	$2*10^{9}$-$2*10^{12}$ vp	nasal swabs	1 week	-	(69)
			2 weeks	-	(69)
			4 weeks	-	(69)
		serum	1 week	-	(69)
			2 weeks	-	(69)
			4 weeks	-	(69)
		urine	1 week	-	(69)
			2 weeks	-	(69)

			4 weeks	-	(69)
			6 weeks	-	(69)
human	$2*10^{10}$-$2*10^{12}$ vp	injection site	1 day	+	(151)
			2 days	+	(151)
			4 days	+	(151)
			30 days	+	(151)
		plasma	30 minutes	+	(65)
			24 hours	+	(65)
human	$2.5*10^{11}$ - $5.6*10^{12}$ vp	blood	24 hours	-	(70)
		nasal swab	24 hours	-	(70)
		urine	24 hours	-	(70)
human	$1*10^{6}$ pfu	saliva	0-24 hours	+	(59)
			9 days	-	(59)
		sputum	0-24 hours	+	(59)
			9 days	-	(59)
		urine	0-24 hours	+	(59)
			9 days	-	(59)
human	$1*10^{7}$ pfu	blood	1 day	+	(62)
		blood lymphocytes, peripheral	0 days	+	(60)
		bronchial fluid	0 days	+	(60)
			8 days	+	(60)
			15 days	+	(60)
			30 days	+	(60)
			60 days	-	(60)
		faeces	2 days	-	(60)

		plasma	0 days	+	(60)
			2 days	+	(60)
			7 days	+	(60)
		sputum	2 days	+	(60)
			4-5 days	+	(60)
			11 days	+	(60)
			5-13 days	+	(62)
		throat swabs	2 days	-	(60)
		tumour biopsy	8 days	+	(62)
			1 month	+	(62)
			2 months	+	(62)
			3 months	+	(62)
		urine	2 days	-	(60)
human	$1*10^8$ pfu	blood	1 day	+	(62)
		blood lymphocytes, peripheral	0 days	+	(60)
		bronchial fluid	0 days	+	(60)
			8 days	+	(60)
			15 days	+	(60)
			30 days	+	(60)
			60 days	-	(60)
		faeces	2 days	+	(60)
		plasma	30 minutes	+	(59)
			60 minutes	+	(59)
			90 minutes	+	(59)

			0 days	+	(60)
			2 days	-	(60)
		saliva	0-24 hours	+	(59)
			9 days	-	(59)
		sputum	0-24 hours	+	(59)
			2 days	+	(60)
			9 days	-	(59)
			13 days	+	(60)
			5-13 days	+	(62)
		throat swabs	2 days	-	(60)
			9-11 days	+	(60)
		tumour biopsy	8 days	+	(62)
			1 month	+	(62)
			2 months	+	(62)
			3 months	+	(62)
		urine	0-24 hours	+	(59)
			2 days	-	(60)
			9 days	-	(59)
mice	$2*10^8$ pfu	liver	24 hours	+	(58)
		tumour	24 hours	+	(58)
mice	$3*10^8$ pfu	blood	25 seconds	+	(58)
			50 seconds	+	(58)
			5 minutes	+	(58)
			10 minutes	+	(58)
			2 hours	+	(58)
			24 hours	-	(58)

		liver	10 minutes	+	(58)
		tumour	10 minutes	+	(58)
human	$1*10^9$ pfu	blood lymphocytes, peripheral	0 days	+	(60)
			2 days	+	(60)
			4 days	+	(60)
		bronchial fluid	0 days	+	(60)
			8-10 days	+	(60)
			12-15 days	+	(60)
			30 days	+	(60)
			60 days	+	(60)
			90 days	+	(60)
		faeces	2 days	+	(60)
		plasma	30 minutes	+	(59)
			60 minutes	+	(59)
			90 minutes	+	(59)
			0 days	+	(60)
			2 days	+	(60)
			4 days	+	(60)
		saliva	0-24 hours	+	(59)
			9 days	-	(59)
		sputum	0-24 hours	+	(59)
			2 days	+	(60)
			3-4 days	+	(60)
			8-10 days	+	(60)
			9 days	-	(59)
			12-15 days	+	(60)
			30 days	+	(60)

			60 days	+	(60)
			90 days	-	(60)
		throat swabs	2 days	+	(60)
		urine	0-24 hours	+	(59)
			2 days	-	(60)
			9 days	-	(59)
human	$3*10^9$ pfu	plasma	30 minutes	+	(59)
			60 minutes	+	(59)
			90 minutes	+	(59)
		saliva	0-24 hours	+	(59)
			9 days	-	(59)
		sputum	0-24 hours	+	(59)
			9 days	-	(59)
		urine	0-24 hours	+	(59)
			9 days	-	(59)
human	$1*10^{10}$ pfu	plasma	30 minutes	+	(59)
			60 minutes	+	(59)
			90 minutes	+	(59)
		saliva	0-24 hours	+	(59)
			9 days	-	(59)
		sputum	0-24 hours	+	(59)
			9 days	-	(59)
		urine	0-24 hours	+	(59)
			9 days	-	(59)

human	$3*10^{10}$ pfu	plasma	30 minutes	+	(59)
			60 minutes	+	(59)
			90 minutes	+	(59)
		saliva	0-24 hours	+	(59)
			9 days	-	(59)
		sputum	0-24 hours	+	(59)
			9 days	-	(59)
		urine	0-24 hours	+	(59)
			9 days	-	(59)
human	$1*10^{11}$ pfu	plasma	30 minutes	+	(59)
			60 minutes	+	(59)
			90 minutes	+	(59)
		saliva	0-24 hours	+	(59)
			9 days	-	(59)
		sputum	0-24 hours	+	(59)
			9 days	-	(59)
		urine	0-24 hours	+	(59)
			9 days	-	(59)
human	$1*10^{6}$-$1*10^{11}$ pfu	blood	30 minutes	+	(68)
			90 minutes	+	(68)
			24 hours	+	(68)
			48 hours	-	(68)
		saliva	1-7 days	+	(68)
			> 7 days	-	(68)

		sputum	1-7 days	+	(68)
			> 7 days	-	(68)
		urine	1 day	+	(68)
			3-17 days	+	(68)
			> 17 days	-	(68)
human	$1*10^{7}$-$1*10^{10}$ pfu	blood	24 hours	+	(64)
		stool	24 hours	-	(64)
			7 days	-	(64)
		throat swab	24 hours	-	(64)
			7 days	-	(64)
		tumour biopsy	7 days	+	(64)
		urine	24 hours	-	(64)
			7 days	-	(64)
human	$1*10^{9}$-$1*10^{11}$ pfu	gargle	1 day	+	(61)
			2 days	+	(61)
			3 days	+	(61)
			4 days	+	(61)
			5 days	+	(61)
			6 days	+	(61)
			7 days	+	(61)
			8 days	+	(61)
			9 days	+	(61)
			10 days	+	(61)
			11 days	+	(61)
			12 days	+	(61)
			13 days	+	(61)
			14 days	+	(61)
			15 days	+	(61)

kidney	25 days	-	(61)
	151 days	-	(61)
liver	25 days	-	(61)
	151 days	-	(61)
lymph node, distal	25 days	-	(61)
	151 days	-	(61)
lymph node, proximal	25	+	(61)
	151 days	+	(61)
plasma	30 minutes	+	(61)
testis	25 days	-	(61)
	151 days	-	(61)
tumour	25	+	(61)
	151 days	+	(61)
urine	1 day	-	(61)
	2 days	-	(61)
	3 days	+	(61)
	4 days	+	(61)
	5 days	+	(61)
	6 days	+	(61)
	7 days	+	(61)
	8 days	-	(61)
	9 days	+	(61)
	10 days	+	(61)
	11 days	+	(61)
	12 days	+	(61)

	13 days	+	(61)
	14 days	+	(61)
	15 days	-	(61)

vp = vector particles
pfu = plaque forming units
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.4. Dermal administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
rabbit	$7*10^{10}$ vp (4 weekly dosages)	blood	22 days	-	(71)
		brain	22 days	-	(71)
		gonads	22 days	-	(71)
		heart	22 days	-	(71)
		kidney	22 days	-	(71)
		liver	22 days	-	(71)
		lung	22 days	-	(71)
		lymph node, axillary	22 days	+	(71)
		lymph node, mesenteric cranial	22 days	-	(71)
		lymph node, mesenteric posterior	22 days	-	(71)
		spleen	22 days	-	(71)
		wound bed	22 days	+	(71)
rabbit	$7*10^{11}$ vp (4 weekly dosages)	blood	22 days	-	(71)
		brain	22 days	-	(71)
		gonads	22 days	-	(71)

	heart	22 days	-	(71)
	kidney	22 days	-	(71)
	liver	22 days	-	(71)
	lung	22 days	-	(71)
	lymph node, axillary	22 days	+	(71)
	lymph node, mesenteric cranial	22 days	-	(71)
	lymph node, posterior mesenteric	22 days	-	(71)
	spleen	22 days	-	(71)
	wound bed	22 days	+	(71)

vp = vector particles
pfu = plaque forming units
iu = infectious units
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.5. Intra-prostatic administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
dog	$3*10^{8}$ - $1*10^{12}$ vp	bladder	1-5 days	+	(74)
			2 days	+	(74)
			7 days	+	(74)
		duodenum	1-5 days	+	(74)
			7 days	+	(74)
		epididymus	2 days	+	(74)
		heart	2 days	+	(74)
		kidney	2 days	+	(74)
		liver	2 days	+	(74)
		lung	2 days	+	(74)
		muscle	2 days	+	(74)
		prostate	1-5 days	+	(74)
			2 days	+	(74)
			7 days	-	(74)
		rectum	2 days	+	(74)
		salivary gland	1-5 days	+	(74)
			7 days	+	(74)
		spleen	2 days	+	(74)

		stomach	1-5 days	+	(74)
			2 days	+	(74)
			7 days	+	(74)
		testis	2 days	+	(74)
		thyroid	1-5 days	+	(74)
			2 days	+	(74)
			7 days	+	(74)
human	$1*10^{12}$ vp	blood	45 days	+	(73)
			>45 days	-	(73)
mice	$1*10^{8}$ pfu	kidney	14 days	+	(39)
		liver	14 days	+	(39)
		lung	14 days	+	(39)
		prostate	14 days	+	(39)
		spleen	14 days	+	(39)
human	$2.5*10^{8}$ pfu	serum	2 days	-	(54)
		urine	2 days	+	(54)
			3 days	-	(54)
human	$1*10^{9}$ pfu	blood	1 month	-	(72)
		prostate	1 month	+	(72)
		saliva	1 month	-	(72)
		stool	1 month	-	(72)

human	$2.5*10^9$ pfu	serum	2 days	-	(54)
		urine	2 days	+	(54)
			3-10 days	-	(54)
human	$1*10^{10}$ pfu	blood	1 month	-	(72)
		prostate	1 month	+	(72)
		saliva	1 month	-	(72)
		stool	1 month	-	(72)
human	$1*10^8$ iu	ear swab	1-4 days	-	(75)
		nasal swab	1-4 days	-	(75)
		serum	1-4 days	-	(75)
		urine	1-4 days	-	(75)
human	$1*10^9$ iu	ear swab	1-4 days	-	(75)
		nasal swab	1-4 days	-	(75)
		serum	1-4 days	-	(75)
		urine	1 day	+	(75)
			2-5 days	-	(75)
human	$1*10^{10}$ iu	ear swabs	1-4 days	-	(75)
		nasal swab	1-4 days	-	(75)
		serum	1-4 days	-	(75)

		urine	1-11 days	+	(75)
			12-15 days	-	(75)
human	$1*10^{11}$ iu	ear swab	1-4 days	-	(75)
		nasal swab	1-4 days	-	(75)
		serum	1-4 days	-	(75)
		urine	1-32 days	+	(75)
			33-36 days	-	(75)

vp = vector particles
pfu = plaque forming units
iu = infectious units ($1*10^{11}$ iu/ml = $2*10^{12}$ vp/ml (75))
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.6. Brain administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
human	$2*10^{9}$-$2*10^{12}$ vp	nasal swabs	1 week	-	(69)
			2 weeks	-	(69)
			4 weeks	-	(69)
		serum	1 week	-	(69)
			2 weeks	-	(69)
			4 weeks	-	(69)
		urine	1 week	-	(69)
			2 weeks	-	(69)
			4 weeks	-	(69)
			6 weeks	-	(69)
human	$4.6*10^{8}$ vp	blood	1-2 days	-	(76)
		faeces	1-2 days	-	(76)
		nasal swab	1-2 days	-	(76)
		urine	1-2 days	-	(76)
human	$4.6*10^{9}$ vp	blood	1-2 days	-	(76)
		faeces	1-2 days	-	(76)
		nasal swab	1-2 days	-	(76)
		urine	1-2 days	-	(76)
human	$3*10^{10}$ vp	plasma	24 hours	-	(77)
			2 weeks	-	(77)
			1 month	-	(77)

		rectal sample	24 hours	-	(77)
			2 weeks	-	(77)
			1 month	-	(77)
		sputum	24 hours	-	(77)
			2 weeks	-	(77)
			1 month	-	(77)
		urine	24 hours	-	(77)
			2 weeks	-	(77)
			1 month	-	(77)
human	$4.6*10^{10}$ vp	blood	1-2 days	-	(76)
		faeces	1-2 days	-	(76)
		nasal swab	1-2 days	-	(76)
		urine	1-2 days	-	(76)
human	$2.5*10^{11}$ vp	blood	24 hours	-	(70)
		nasal swab	24 hours	-	(70)
		urine	24 hours	-	(70)
human	$3*10^{11}$ vp	blood	24 hours	-	(70)
		nasal swab	24 hours	-	(70)
		plasma	24 hours	-	(77)
			2 weeks	-	(77)
			1 month	-	(77)
		rectal sample	24 hours	-	(77)

			2 weeks	-	(77)
			1 month	-	(77)
		sputum	24 hours	-	(77)
			2 weeks	-	(77)
			1 month	-	(77)
		urine	24 hours	-	(70)
			24 hours	-	(77)
			2 weeks	-	(77)
			1 month	-	(77)
human	$4.6*10^{11}$ vp	blood	1-2 days	-	(76)
		faeces	1-2 days	-	(76)
		nasal swab	1-2 days	-	(76)
		urine	1-2 days	-	(76)
human	$9*10^{11}$ vp	blood	24 hours	-	(70)
		nasal swab	24 hours	-	(70)
		urine	24 hours	-	(70)
human	$1*10^{12}$ vp	plasma	24 hours	-	(77)
			2 weeks	-	(77)
			1 month	-	(77)
		rectal sample	24 hours	-	(77)
			2 weeks	-	(77)
			1 month	-	(77)
		sputum	24 hours	-	(77)
			2 weeks	-	(77)

			1 month	-	(77)
		urine	24 hours	-	(77)
			2 weeks	-	(77)
			1 month	-	(77)
human	$2.7*10^{12}$ vp	blood	24 hours	-	(70)
		nasal swab	24 hours	-	(70)
		urine	24 hours	-	(70)
human	$3*10^{12}$ vp	plasma	24 hours	-	(77)
			2 weeks	-	(77)
			1 month	-	(77)
		rectal sample	24 hours	-	(77)
			2 weeks	-	(77)
			1 month	-	(77)
		sputum	24 hours	-	(77)
			2 weeks	-	(77)
			1 month	-	(77)
		urine	24 hours	-	(77)
			2 weeks	-	(77)
			1 month	-	(77)
human	$5.6*10^{12}$ vp	blood	24 hours	-	(70)
		nasal swab	24 hours	-	(70)
		urine	24 hours	-	(70)

human	$3*10^8 – 3*10^{10}$ pfu	plasma	3 days	-	(78)
			5 days	-	(78)
			7 days	-	(78)
			21 days	-	(78)
		urine	3 days	-	(78)
			5 days	-	(78)
			7 days	-	(78)
			21 days	-	(78)
human	$3*10^{10}$ pfu	plasma	3 days	+	(149)

vp = vector particles
pfu = plaque forming units
iu = infectious units
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.7. Intra-muscular administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
human	$2.87*10^8$ vp	blood	2 days	-	(81)
			7 days	-	(81)
			2 weeks	-	(81)
			4 weeks	-	(81)
			8 weeks	-	(81)
			12 weeks	-	(81)
		faeces	2 days	-	(81)
			7 days	-	(81)
			2 weeks	-	(81)
			4 weeks	-	(81)
			8 weeks	-	(81)
			12 weeks	-	(81)
		throat swabs	2 days	-	(81)
			7 days	-	(81)
			2 weeks	-	(81)
			4 weeks	-	(81)
			8 weeks	-	(81)
			12 weeks	-	(81)
		urine	2 days	-	(81)
			7 days	-	(81)
			2 weeks	-	(81)
			4 weeks	-	(81)
			8 weeks	-	(81)
			12 weeks	-	(81)

human	$2.87*10^9$ vp	blood	2 days	-	(81)
			7 days	-	(81)
			2 weeks	-	(81)
			4 weeks	-	(81)
			8 weeks	-	(81)
			12 weeks	-	(81)
		faeces	2 days	-	(81)
			7 days	-	(81)
			2 weeks	-	(81)
			4 weeks	-	(81)
			8 weeks	-	(81)
			12 weeks	-	(81)
		throat swabs	2 days	-	(81)
			7 days	-	(81)
			2 weeks	-	(81)
			4 weeks	-	(81)
			8 weeks	-	(81)
			12 weeks	-	(81)
		urine	2 days	-	(81)
			7 days	-	(81)
			2 weeks	-	(81)
			4 weeks	-	(81)
			8 weeks	-	(81)
			12 weeks	-	(81)
human	$2.87*10^{10}$ vp	blood	2 days	-	(81)
			7 days	-	(81)
			2 weeks	-	(81)
			4 weeks	-	(81)
			8 weeks	-	(81)

			12 weeks	-	(81)
		faeces	2 days	-	(81)
			7 days	-	(81)
			2 weeks	-	(81)
			4 weeks	-	(81)
			8 weeks	-	(81)
			12 weeks	-	(81)
		throat swabs	2 days	-	(81)
			7 days	-	(81)
			2 weeks	-	(81)
			4 weeks	-	(81)
			8 weeks	-	(81)
			12 weeks	-	(81)
		urine	2 days	-	(81)
			7 days	-	(81)
			2 weeks	-	(81)
			4 weeks	-	(81)
			8 weeks	-	(81)
			12 weeks	-	(81)
pig	$1*10^{12}$ vp	liver	24 hours	+	(51)
		lung	24 hours	+	(51)
		spleen	24 hours	+	(51)
		ventricle, left	1 hour	+	(51)
			24 hours	+	(51)

pig	$1.5*10^9$ pfu	stool	2 days	-	(82)
			7 days	-	(82)
		urine	2 days	-	(82)
			7 days	-	(82)
human	$6.4*10^6$ iu ($3.3*10^8$ vp)	blood, pulmonary arterial	0 minutes	+	(80)
		blood, venous	1 hour	-	(80)
		semen	8 weeks	-	(80)
		urine	?	-	(80)
human	$2.0*10^7$ iu ($1*10^9$ vp)	blood, pulmonary arterial	0 minutes	+	(80)
		blood, venous	1 hour	-	(80)
		semen	8 weeks	-	(80)
		urine	?	-	(80)
human	$1.3*10^8$ iu ($3.3*10^9$ vp)	blood, pulmonary arterial	0 minutes	+	(80)
		blood, venous	1 hour	+	(80)
		semen	8 weeks	-	(80)
		urine	?	-	(80)
human	$1.9*10^8$ iu ($1*10^{10}$ vp)	blood, pulmonary arterial	0 minutes	+	(80)
		blood, venous	1 hour	+	(80)
		semen	8 weeks	-	(80)

		urine	?	-	(80)
human	$8.8*10^8$ iu ($3.3*10^{10}$ vp)	blood, pulmonary arterial	0 minutes	+	(80)
		blood, venous	1 hour	+	(80)
		semen	8 weeks	-	(80)
		urine	?	-	(80)

vp = vector particles
pfu = plaque forming units
iu = infectious units
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.8. Inhalatory and nasal-bronchial administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
human	$2.1*10^{9} – 2.1*10^{11}$ vp	bronchial brush sample	4 days	+	(85)
		nasopharyngeal swab	1 day	-	(85)
			2 days	-	(85)
		rectal swab	1 day	-	(85)
			2 days	-	(85)
		sputum	1 day	-	(85)
			2 days	-	(85)
		urine	1 day	-	(85)
			2 days	-	(85)
human	$1.01*10^{7}$ pfu^	blood	1 day	-	(83)
			3 days	-	(83)
			7 days	-	(83)
			14 days	-	(83)
			21 days	-	(83)
			28 days	-	(83)
		bronchial brush	3 days	-	(83)
			7/8 days	+	(83)
			14/15 days	-	(83)
			21/22 days	-	(83)
			28 days	-	(83)
		bronchoalveolar lavage	3 days	+	(83)
			7/8 days	-	(83)
			14/15 days	-	(83)

			21/22 days	-	(83)
			28 days	-	(83)
		nasal brush	1 day	+	(83)
			3 days	-	(83)
			7/8 days	-	(83)
			14/15 days	-	(83)
			21/22 days	+	(83)
			28 days	-	(83)
		saliva	1 day	+	(83)
			2 days	-	(83)
			3 days	-	(83)
			4 days	-	(83)
			7/8 days	+	(83)
			9 days	+	(83)
			14/15 days	-	(83)
			21/22 days	-	(83)
			28 days	-	(83)
		tonsils	1 day	-	(83)
			2 days	-	(83)
			3 days	-	(83)
			4 days	-	(83)
			7/8 days	-	(83)
			9 days	-	(83)
			14/15 days	-	(83)
			21/22 days	-	(83)
			28 days	-	(83)
human	$2*10^7$ pfu	nostril, control	1 to 4 days	-	(84)
		nostril, dosed	2 to 8 days	-	(84)

		pharynx swab	1 to 4 days	-	(84)
		urine	1 to 4 days	+ (wild type)	(84)
		rectal swab	1 to 4 days	-	(84)
human	$1.1*10^8$ pfu^	blood	1 day	+	(83)
			2 days	+	(83)
			3 days	-	(83)
			7 days	-	(83)
			14 days	-	(83)
			21 days	-	(83)
			28 days	-	(83)
		bronchial brush	3 days	-	(83)
			4 days	+	(83)
			7/8 days	+	(83)
			14/15 days	-	(83)
			21 days	-	(83)
			28 days	-	(83)
		bronchoalveolar lavage	3 days	+	(83)
			4 days	+	(83)
			7/8 days	+	(83)
			14/15 days	-	(83)
			21 days	-	(83)
			28 days	-	(83)
		nasal brush	1 day	+	(83)
			3 days	+	(83)
			4 days	+	(83)
			7/8 days	-	(83)
			14/15 days	+	(83)
			21/22 days	-	(83)

			28 days	-	(83)
		saliva	1 day	+	(83)
			2 days	+	(83)
			3 days	-	(83)
			4 days	+	(83)
			7/8 days	+	(83)
			14/15 days	-	(83)
			21/22 days	-	(83)
			28 days	-	(83)
		tonsils	1 day	-	(83)
			2 days	-	(83)
			3 days	-	(83)
			4 days	+	(83)
			7/8 days	-	(83)
			14/15 days	-	(83)
			21/22 days	-	(83)
			28 days	-	(83)
human	$2*10^8$ pfu	nostril, control	1 to 4 days	+	(84)
		nostril, dosed	2 to 8 days	-	(84)
		pharynx swab	1 to 4 days	-	(84)
		urine	1 day	+ (wild type)	(84)
			2 – 4 days	-	(84)
		rectal swab	1 to 4 days	-	(84)

human	$9.4*10^8$ pfu^	blood	1 day	-	(83)
			3 days	-	(83)
			7 days	-	(83)
			14 days	-	(83)
			21 days	-	(83)
			28 days	-	(83)
		bronchial brush	3 days	-	(83)
			7/8 days	-	(83)
			14/15 days	+	(83)
			21 days	-	(83)
			28 days	-	(83)
		bronchoalveolar lavage	3 days	+	(83)
			7/8 days	+	(83)
			14/15 days	-	(83)
			21 days	-	(83)
			28 days	-	(83)
		nasal brush	1 day	+	(83)
			3 days	+	(83)
			7/8 days	+	(83)
			14/15 days	+	(83)
			21/22 days	-	(83)
			28 days	-	(83)
		saliva	1 day	+	(83)
			2 days	-	(83)
			3 days	-	(83)
			4 days	-	(83)
			7/8 days	-	(83)
			9 days	-	(83)

			14/15 days	-	(83)
			21/22 days	-	(83)
			28 days	-	(83)
		tonsils	1 day	+	(83)
			2 days	-	(83)
			3 days	-	(83)
			4 days	-	(83)
			7/8 days	-	(83)
			9 days	-	(83)
			14/15 days	-	(83)
			21/22 days	-	(83)
			28 days	-	(83)
mice	$1*10^9$ pfu	lung	10 minutes	+	(86)
			2 hours	+	(86)
			8 hours	+	(86)
			24 hours	+	(86)
human	$2*10^9$ pfu	nostril, control	1 to 4 days	-	(84)
		nostril, dosed	2 to 8 days	+	(84)
		pharynx swab	1 to 2 days	+	(84)
			3 to 4 days	-	(84)
		urine	1 to 4 days	+ (wild type)	(84)
		rectal swab	1 to 4 days	-	(84)

human	$2*10^{10}$ pfu	nostril, control	1 to 4 days	+	(84)
		nostril, dosed	2 to 8 days	+	(84)
		pharynx swab	1 to 2 days	+	(84)
			3 to 4 days	-	(84)
		urine	1 to 4 days	+ (wild type)	(84)
		rectal swab	1 day	+	(84)
			2 days	+	(84)
			3 to 4 days	-	(84)

vp = vector particles
pfu = plaque forming units
iu = infectious units
^ total dose of 2 different dosages given on Day 0 (nasal instilation) and Day 1 (lung aerosolation).
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.9. Ocular administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
human	$1*10^{6}$ vp	sputum	3 weeks	-	(88)
		urine	3 weeks	-	(88)
human	$1*10^{6,5}$ vp	sputum	3 weeks	-	(88)
		urine	3 weeks	-	(88)
human	$1*10^{7}$ vp	sputum	3 weeks	-	(88)
		urine	3 weeks	-	(88)
human	$1*10^{7,5}$ vp	sputum	3 weeks	-	(88)
		urine	3 weeks	-	(88)
human	$1*10^{8}$ vp	sputum	3 weeks	-	(88)
		urine	3 weeks	-	(88)
human	$1*10^{8,5}$ vp	sputum	3 weeks	-	(88)
		urine	3 weeks	-	(88)
human	$1*10^{9}$ vp	sputum	3 weeks	-	(88)
		urine	3 weeks	-	(88)
human	$1*10^{9,5}$ vp	sputum	3 weeks	-	(88)
		urine	3 weeks	-	(88)

monkey (Cynomolgus)	$7.5*10^{10}$ vp	blood	15 minutes	+	(87)
			1 hour	+	(87)
			2 hours	+	(87)
			20-29 hours	+	(87)
			4 days	+	(87)
			7 days	+	(87)
			29 days	-	(87)
		bone marrow	6 days	+	(87)
			29 days	-	(87)
		brain, occipital	6 days	-	(87)
		conjunctiva/sclera left	6 days	+	(87)
		conjunctiva/sclera right	6 days	+	(87)
		heart	6 days	+	(87)
		kidney	6 days	+	(87)
		lateral geniculate nucleus, left	6 days	-	(87)
		lateral geniculate nucleus, right	6 days	+	(87)
		liver	6 days	+	(87)
			29 days	+	(87)
		lung	6 days	-	(87)
		optic chiasm	6 days	-	(87)

	optic nerve, left	6 days	+	(87)
	optic nerve, right	6 days	+	(87)
	ovary	6 days	-	(87)
		29 days	-	(87)
	retina, left	6 days	+	(87)
	retina right	6 days	-	(87)
	spleen	6 days	+	(87)
		29 days	+	(87)
	testis	6 days	-	(87)
		29 days	-	(87)

vp = vector particles
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.10. Salivary gland administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
rat	$2.4*10^8$ vp	blood	3 days	-	(89)
			29 days	-	(89)
		gonads	3 days	-	(89)
			29 days	-	(89)
		heart	3 days	-	(89)
			29 days	-	(89)
		liver	3 days	-	(89)
			29 days	-	(89)
		lung	3 days	-	(89)
			29 days	-	(89)
		spleen	3 days	-	(89)
			29 days	+	(89)
		submandibular gland, left	3 days	+	(89)
			29 days	-	(89)
		submandibular gland, right	3 days	+	(89)
			29 days	+	(89)
rat	$6*10^9$ vp	blood	3 days	-	(89)
			29 days	+	(89)
		gonads	3 days	-	(89)
			29 days	-	(89)

		heart	3 days	-	(89)
			29 days	+	(89)
		liver	3 days	+	(89)
			29 days	+	(89)
		lung	3 days	+	(89)
			29 days	+	(89)
		spleen	3 days	+	(89)
			29 days	+	(89)
		submandibular gland, left	3 days	+	(89)
			29 days	+	(89)
		submandibular gland, right	3 days	+	(89)
			29 days	+	(89)
rat	$1.5*10^{11}$ vp	blood	3 days	+	(89)
			29 days	-	(89)
		gonads	3 days	-	(89)
			29 days	+	(89)
		heart	3 days	-	(89)
			29 days	-	(89)
		liver	3 days	+	(89)
			29 days	+	(89)
		lung	3 days	+	(89)
			29 days	+	(89)
		spleen	3 days	+	(89)

			29 days	+	(89)
		submandibular gland, left	3 days	+	(89)
			29 days	+	(89)
		submandibular gland, right	3 days	+	(89)
			29 days	+	(89)
rat	$2*10^{11}$ vp	blood	3 days	-	(90)
			15 days	-	(90)
			29 days	-	(90)
			57 days	-	(90)
			92 days	-	(90)
		brain	3 days	-	(90)
			15 days	-	(90)
			29 days	-	(90)
			57 days	-	(90)
			92 days	-	(90)
		gonads	3 days	-	(90)
			15 days	+	(90)
			29 days	-	(90)
			57 days	-	(90)
			92 days	-	(90)
		heart	3 days	-	(90)
			15 days	-	(90)
			29 days	-	(90)
			57 days	-	(90)
			92 days	-	(90)
		intestine, large	3 days	-	(90)
			15 days	-	(90)

	29 days	-	(90)
	57 days	-	(90)
	92 days	-	(90)
intestine, small	3 days	-	(90)
	15 days	-	(90)
	29 days	-	(90)
	57 days	-	(90)
	92 days	-	(90)
kidney	3 days	-	(90)
	15 days	-	(90)
	29 days	-	(90)
	57 days	-	(90)
	92 days	-	(90)
liver	3 days	+	(90)
	15 days	+	(90)
	29 days	-	(90)
	57 days	-	(90)
	92 days	-	(90)
lung	3 days	+	(90)
	15 days	-	(90)
	29 days	-	(90)
	57 days	-	(90)
	92 days	-	(90)
lymph node, mandibular left	3 days	+	(90)
	15 days	+	(90)
	29 days	-	(90)
	57 days	-	(90)
	92 days	+	(90)

lymph node, mandibular right	3 days	+	(90)
	15 days	-	(90)
	29 days	-	(90)
	57 days	-	(90)
	92 days	+	(90)
oral mucosa, buccal	3 days	-	(90)
	15 days	-	(90)
	29 days	-	(90)
	57 days	-	(90)
	92 days	-	(90)
oral mucosa, floor-of-mouth	3 days	+	(90)
	15 days	-	(90)
	29 days	-	(90)
	57 days	-	(90)
	92 days	-	(90)
oral mucosa, palatal	3 days	-	(90)
	15 days	-	(90)
	29 days	-	(90)
	57 days	-	(90)
	92 days	-	(90)
parotid gland, left	3 days	-	(90)
	15 days	-	(90)
	29 days	-	(90)
	57 days	-	(90)
	92 days	-	(90)
parotid gland, right	3 days	+	(90)
	15 days	-	(90)

		29 days	-	(90)
		57 days	-	(90)
		92 days	-	(90)
	spleen	3 days	+	(90)
		15 days	+	(90)
		29 days	-	(90)
		57 days	-	(90)
		92 days	+	(90)
	sublingual gland, left	3 days	-	(90)
		15 days	+	(90)
		29 days	-	(90)
		57 days	-	(90)
		92 days	+	(90)
	sublingual gland, right	3 days	-	(90)
		15 days	+	(90)
		29 days	-	(90)
		57 days	-	(90)
		92 days	+	(90)
	submandibular gland, left	3 days	+	(90)
		29 days	+	(90)
		57 days	+	(90)
		92 days	+	(90)
	submandibular gland, right	3 days	+	(90)
		15 days	+	(90)
		29 days	+	(90)
		57 days	+	(90)
		92 days	+	(90)

	tongue	3 days	-	(90)
		15 days	-	(90)
		29 days	-	(90)
		57 days	-	(90)
		92 days	-	(90)

vp = vector particles
pfu = plaque forming units
iu = infectious units
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.11. Para-aortic lymph node administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
human	$2.5*10^8$ pfu	serum	2 days	-	(54)
		urine	2 days	-	(54)
human	$2.5*10^{10}$ pfu	serum	2 days	+	(54)
			3 days	+	(54)
			7 to 10 days	-	(54)
		urine	2 days	-	(54)
			3 days	-	(54)
			7 to 10 days	-	(54)

pfu = plaque forming units
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.12. Spinal administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
human	$2.5*10^{8}$ pfu	serum	2 days	-	(54)
		urine	2 days	-	(54)
human	$2.5*10^{10}$ pfu	serum	2 days	+	(54)
			3 days	+	(54)
			7 to 10 days	-	(54)
		urine	2 to 10 days	-	(54)

pfu = plaque forming units
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.13. Ileum administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
human	$2.5*10^{9}$ pfu	serum	2 days	-	(54)
		urine	2 to 10 days	-	(54)
human	$2.5*10^{10}$ pfu	serum	2 days	+	(54)
			3 to 10 days	-	(54)
		urine	2 to 10 days	-	(54)

pfu = plaque forming units
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.14. Bladder administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
mice	$5*10^9$ pfu	adrenal gland	1 day	+	(50)
		bladder	1 day	+	(50)
			3 days	+	(50)
			7 days	+	(50)
			14 days	+	(50)
			21 days	+	(50)
		gonads	1 day	+	(50)
		heart	1 day	+	(50)
		kidney	1 day	+	(50)
		liver	1 day	+	(50)
		lung	1 day	+	(50)
		ureter	1 day	+	(50)

pfu = plaque forming units
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.15. foetal administration of HAdV-5

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
administration to the amniotic fluid					
mice	$3*10^6$ pfu	digestive epithelium	72 hours	+	(91)
		lung		+	(91)
		skin surface		+	(91)
administration to the intra-peritoneal space					
mice	$3*10^6$ pfu	peritoneum	72 hours	+	(91)
		digestive tract, upper part	72 hours	+	(91)
administration to the placental parenchyma					
mice	$3*10^6$ pfu	foetal organs	72 hours	-	(91)
administration to the umbilical vein					
guinea pig	$3*10^8$ pfu	adrenal gland	24 hours	+	(91)
		brainstem	24 hours	+	(91)
		cerebral cortex	24 hours	-	(91)
		heart	24 hours	+	(91)
		intestine, small	24 hours	+	(91)
		kidney	24 hours	+	(91)
		liver	24 hours	+	(91)
		lung	24 hours	+	(91)

	muscle	24 hours	+	(91)
	placenta	24 hours	+	(91)
	spleen	24 hours	+	(91)

pfu = plaque forming units
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.16. Intravenous administration of AAV2

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
monkey (Macaque)	$5*10^{10}$ vp	heart	22 weeks	-	(98)
		kidney	22 weeks	-	(98)
		liver	22 weeks	+	(98)
		lung	22 weeks	-	(98)
		spleen	22 weeks	-	(98)
mice	$2.1*10^{10}$-$8.4*10^{10}$ vp	liver	1 day	+	(96)
			3 days	+	(96)
			7 days	+	(96)
			35 days	+	(96)
			49 days	+	(152)
			91 days	-	(96)
		muscle	1 day	+	(96)
			3 days	-	(96)
			7 days	-	(96)
			35 days	-	(96)
			91 days	-	(96)
		spleen	1 day	+	(96)
			3 days	+	(96)
			7 days	-	(96)
			35 days	-	(96)
			91 days	-	(96)

monkey (Macaque)	$1*10^{11}$ vp	heart	22 weeks	+	(98)
		kidney	22 weeks	-	(98)
		liver	22 weeks	+	(98)
		lung	22 weeks	-	(98)
		spleen	22 weeks	+	(98)
mice	$2*10^{11}$ vp	brain	6 weeks	+	(97)
		heart	6 weeks	+	(97)
		kidney	6 weeks	+	(97)
		liver	6 weeks	+	(97)
		lung	6 weeks	+	(97)
		spleen	6 weeks	+	(97)
rat	$1.0*10^{11}$ vp/kg	gonads	50 days	+	(93)
			92 days	-	(93)
rabbit	$1.0*10^{11}$ vp/kg	gonads	18-20 months	-	(95)
		liver	18-20 months	+	(95)
		semen	4 days	+	(95)
			1 week	+	(95)
			2 weeks	-	(95)
			3 weeks	+	(95)
			4 weeks	-	(95)
			6 weeks	+	(95)

			8 weeks	-	(95)
			13 weeks	-	(95)
			18 weeks	-	(95)
			23 weeks	-	(95)
			28 weeks	-	(95)
			12 months	-	(95)
			18 months	-	(95)
rat	$1.0*10^{12}$ vp/kg	gonads	50 days	+	(93)
			92 days	-	(93)
rabbit	$1.0*10^{12}$ vp/kg	gonads	18-20 months	+	(95)
		liver	18-20 months	+	(95)
		semen	4 days	+	(95)
			1 week	+	(95)
			2 weeks	+	(95)
			3 weeks	+	(95)
			4 weeks	+	(95)
			6 weeks	+	(95)
			8 weeks	+	(95)
			13 weeks	-	(95)
			18 weeks	-	(95)
			23 weeks	-	(95)
			28 weeks	-	(95)
			12 months	-	(95)
			18 months	-	(95)
dog	$3.7*10^{12} – 7.0*10^{12}$ vp/kg	gonads	90 days	-	(93)
		semen	7 days	-	(93)
			30days	-	(93)
			60 days	-	(93)
			90 days	-	(93)

monkey (Macaque)	$4.0*10^{12}$ vp/kg	gonads	11 months	-	(100)
		kidneys	11 months	-	(100)
		liver	11 months	+	(100)
		plasma	3 days	+	(100)
			6 days	+	(100)
			8 days	-	(100)
		saliva	3 days	+	(100)
			6 days	-	(100)
			8 days	-	(100)
		spleen	11 months	+	(100)
		urine	3 days	+	(100)
			6 days	+	(100)
			8 days	-	(100)
rat	$5*10^{12}$ vp/kg	liver	7 weeks	+	(94)
rat	$1.0*10^{13}$ vp/kg	gonads	50 days	+	(93)
			92 days	+	(93)
rabbit	$1.0*10^{13}$ vp/kg	gonads	18-20 months	+	(95)
		liver	18-20 months	+	(95)
		semen	4 days	+	(95)
			1 week	+	(95)
			2 weeks	+	(95)
			3 weeks	+	(95)
			4 weeks	+	(95)
			6 weeks	+	(95)

			8 weeks	+	(95)
			13 weeks	+	(95)
			18 weeks	-	(95)
			23 weeks	-	(95)
			28 weeks	-	(95)
			12 months	-	(95)
			18 months	-	(95)
monkey (Cynomolgus)	$1.0*10^{10}$ vp/kg	adrenal gland	3 months	-	(101)
			7 months	-	(101)
		bladder	3 months	+	(101)
			7 months	-	(101)
		bone marrow	3 months	-	(101)
			7 months	-	(101)
		cerebellum	3 months	-	(101)
			7 months	-	(101)
		cerebrum	3 months	-	(101)
			7 months	-	(101)
		colon	3 months	+	(101)
			7 months	+	(101)
		epididymis	3 months	-	(101)
			7 months	-	(101)
		gallbladder	3 months	-	(101)
			7 months	-	(101)
		gonads	3 months	-	(101)
			7 months	-	(101)

heart	3 months	-	(101)
	7 months	-	(101)
ileum	3 months	+	(101)
	7 months	-	(101)
jejunum	3 months	-	(101)
	7 months	-	(101)
kidney	3 months	-	(101)
	7 months	-	(101)
liver	3 months	+	(101)
	7 months	-	(101)
lung	3 months	-	(101)
	7 months	-	(101)
lymph node, axillary	3 months	+	(101)
	7 months	+	(101)
lymph node, hilar	3 months	+	(101)
	7 months	+	(101)
lymph node, iliac	3 months	+	(101)
	7 months	+	(101)
lymph node, inguinal	3 months	+	(101)
	7 months	-	(101)
lymph node, mesenteric	3 months	+	(101)
	7 months	+	(101)

muscle	3 months	-	(101)
	7 months	-	(101)
oesophagus	3 months	-	(101)
	7 months	-	(101)
pancreas	3 months	-	(101)
	7 months	-	(101)
parotid gland	3 months	-	(101)
	7 months	-	(101)
retina	3 months	-	(101)
	7 months	+	(101)
skin	3 months	-	(101)
	7 months	-	(101)
spleen	3 months	+	(101)
	7 months	+	(101)
stomach	3 months	-	(101)
	7 months	-	(101)
submandibular gland	3 months	+	(101)
	7 months	-	(101)
thymus	3 months	-	(101)
	7 months	-	(101)
thyroid	3 months	-	(101)
	7 months	-	(101)

		tonsil	3 months	+	(101)
			7 months	-	(101)
		trachea	3 months	-	(101)
			7 months	-	(101)
		uterus	3 months	-	(101)
			7 months	-	(101)
monkey (Cynomolgus)	$2.5*10^{10}$ vp/kg	adrenal gland	2 days	-	(101)
			3 months	-	(101)
		bladder	2 days	+	(101)
			3 months	+	(101)
		bone marrow	2 days	+	(101)
			3 months	+	(101)
		cerebellum	2 days	+	(101)
			3 months	+	(101)
		cerebrum	2 days	+	(101)
			3 months	+	(101)
		colon	2 days	+	(101)
			3 months	+	(101)
		epididymis	2 days	+	(101)
			3 months	+	(101)
		gallbladder	2 days	+	(101)
			3 months	+	(101)

	gonads	2 days	+	(101)
		3 months	+	(101)
	heart	2 days	-	(101)
		3 months	+	(101)
	ileum	2 days	+	(101)
		3 months	+	(101)
	kidney	2 days	-	(101)
		3 months	+	(101)
	liver	2 days	+	(101)
		3 months	+	(101)
	lung	2 days	+	(101)
		3 months	+	(101)
	lymph node, axillary	2 days	+	(101)
		3 months	+	(101)
	lymph node, hilar	2 days	+	(101)
		3 months	+	(101)
	lymph node, iliac	2 days	+	(101)
	lymph node, inguinal	2 days	+	(101)
		3 months	+	(101)
	lymph node, mesenteric	2 days	+	(101)
		3 months	+	(101)
	muscle	2 days	-	(101)

		3 months	-	(101)
	oesophagus	2 days	+	(101)
		3 months	-	(101)
	pancreas	2 days	+	(101)
		3 months	+	(101)
	parotid gland	2 days	+	(101)
		3 months	-	(101)
	retina	2 days	-	(101)
		3 months	-	(101)
	skin	2 days	-	(101)
		3 months	+	(101)
	spleen	2 days	+	(101)
		3 months	+	(101)
	stomach	2 days	+	(101)
		3 months	+	(101)
	submandibular gland	2 days	+	(101)
		3 months	-	(101)
	thymus	2 days	+	(101)
		3 months	-	(101)
	thyroid	2 days	+	(101)
		3 months	+	(101)
	tonsil	2 days	+	(101)

			3 months	+	(101)
		trachea	2 days	+	(101)
			3 months	+	(101)
		uterus	2 days	+	(101)
			3 months	+	(101)
monkey (Cynomolgus)	$1.0*10^{11}$ vp/kg	adrenal gland	5 months	+	(101)
		bladder	5 months	+	(101)
		bone marrow	5 months	+	(101)
		cerebellum	5 months	-	(101)
		cerebrum	5 months	-	(101)
		colon	5 months	+	(101)
		epididymis	5 months	+	(101)
		gallbladder	5 months	+	(101)
		gonads	5 months	-	(101)
		heart	5 months	+	(101)
		ileum	5 months	+	(101)
		jejunum	5 months	-	(101)
		kidney	5 months	+	(101)

	liver	5 months	+	(101)
	lung	5 months	+	(101)
	lymph node, axillary	5 months	+	(101)
	lymph node, iliac	5 months	+	(101)
	lymph node, inguinal	5 months	+	(101)
	lymph node, mesenteric	5 months	+	(101)
	muscle	5 months	+	(101)
	oesophagus	5 months	-	(101)
	pancreas	5 months	+	(101)
	parotid gland	5 months	-	(101)
	skin	5 months	-	(101)
	spleen	5 months	+	(101)
	stomach	5 months	-	(101)
	submandibular gland	5 months	+	(101)
	thymus	5 months	+	(101)
	thyroid	5 months	+	(101)
	tonsil	5 months	+	(101)

		trachea	5 months	-	(101)
		uterus	5 months	+	(101)
monkey (Cynomolgus)	$2.5*10^{11}$ vp/kg	adrenal gland	3 months	+	(101)
		bladder	3 months	+	(101)
		bone marrow	3 months	+	(101)
		cerebellum	3 months	-	(101)
		cerebrum	3 months	+	(101)
		colon	3 months	+	(101)
		epididymis	3 months	-	(101)
		gallbladder	3 months	+	(101)
		gonads	3 months	-	(101)
		heart	3 months	+	(101)
		ileum	3 months	-	(101)
		jejunum	3 months	-	(101)
		kidney	3 months	+	(101)
		liver	3 months	+	(101)
		lung	3 months	+	(101)

lymph node, axillary	3 months	+	(101)
lymph node, hilar	3 months	+	(101)
lymph node, iliac	3 months	+	(101)
lymph node, inguinal	3 months	+	(101)
lymph node, mesenteric	3 months	+	(101)
muscle	3 months	+	(101)
oesophagus	3 months	+	(101)
pancreas	3 months	+	(101)
parotid gland	3 months	+	(101)
skin	3 months	-	(101)
spinal cord	3 months	-	(101)
spleen	3 months	+	(101)
stomach	3 months	-	(101)
submandibular gland	3 months	-	(101)
thymus	3 months	-	(101)
thyroid	3 months	-	(101)

	tonsil	3 months	+	(101)
	trachea	3 months	+	(101)
	uterus	3 months	-	(101)

vp = vector particles
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.17. Intraperitoneal administration of AAV2

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
mice	$2*10^{11}$ vp	abdominal tissue	2 months	+	(102)
		diaphragm	2 months	+	(102)
		heart	2 months	-	(102)
		kidney	2 months	-	(102)
		liver	2 months	-	(102)
		lung	2 months	-	(102)
		muscle, intercostal	2 months	+	(102)
		muscle, masseter	2 months	-	(102)
		muscle, triceps	2 months	-	(102)
		spleen	2 months	-	(102)
		testis	2 months	-	(102)

vp = vector particles
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.18. Intra-muscular administration of AAV2

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
mice	$5*10^{10}$ vp	heart	22 weeks	+	(98)
		kidney	22 weeks	+	(98)
		liver	22 weeks	+	(98)
		lung	22 weeks	+	(98)
		spleen	22 weeks	+	(98)
rat	$4.0*10^{11}$ vp	heart	6 months	+	(103)
		kidney	6 months	+	(103)
		liver	6 months	+	(103)
		spleen	6 months	-	(103)
		testes	6 months	+	(103)
mice	$1.0*10^{11}$ vp	liver	56 days	+	(104)
dog	$2.5*10^{12}$ vp	adrenal gland	10 weeks	-	(106)
		brain	10 weeks	-	(106)
		kidney	10 weeks	-	(106)
		liver	10 weeks	-	(106)
		lung	10 weeks	-	(106)

		lymph node, inguinal right	10 weeks	+	(106)
		muscle, control	10 weeks	-	(106)
		muscle, injected	10 weeks	+	(106)
		testis	10 weeks	-	(106)
		thymus	10 weeks	-	(106)
		thyroid	10 weeks	-	(106)
dog	$1.2*10^{13}$ vp	blood, peripheral MNC	33 weeks	-	(105)
		brain	33 weeks	-	(105)
		gonads	33 weeks	-	(105)
		heart	33 weeks	-	(105)
		intestine	33 weeks	-	(105)
		kidney	33 weeks	-	(105)
		liver	33 weeks	+	(105)
		lung	33 weeks	-	(105)
		lymph nodes	33 weeks	-	(105)
		muscle, control	33 weeks	-	(105)
		muscle, injected	33 weeks	+	(105)

		spinal cord	33 weeks	-	(105)
		spleen	33 weeks	-	(105)
human	$1.4*10^{13} – 7.0*10^{14}$ vp	saliva	0 days	+	(25)
			1 day	+	(25)
			2 days	+	(25)
			3 days	-	(25)
			4 days	-	(25)
			7 days	-	(25)
			2 weeks	+	(25)
			3 weeks	-	(25)
			4 weeks	-	(25)
			5 weeks	-	(25)
			6 weeks	-	(25)
			7 weeks	-	(25)
			8 weeks	-	(25)
			10 weeks	-	(25)
			12 weeks	-	(25)
		semen	2 days	-	(25)
			3 days	-	(25)
			4 days	-	(25)
			7 days	-	(25)
			2 weeks	-	(25)
			3 weeks	-	(25)
			4 weeks	-	(25)
			5 weeks	-	(25)
			6 weeks	-	(25)
			7 weeks	-	(25)
			8 weeks	-	(25)
			24 weeks	-	(25)
		serum	0 days	+	(25)

		1 day	+	(25)
		2 days	+	(25)
		4 days	+	(25)
		6 days	-	(25)
		7 days	+	(25)
		2 weeks	-	(25)
		3 weeks	-	(25)
		4 weeks	+	(25)
		5 weeks	-	(25)
		6 weeks	-	(25)
		7 weeks	+	(25)
		8 weeks	-	(25)
		10 weeks	-	(25)
		12 weeks	+	(25)
		14 weeks	-	(25)
		16 weeks	-	(25)
	urine	0 days	+	(25)
		1 day	+	(25)
		2 days	-	(25)
		3 days	-	(25)
		4 days	-	(25)
		6 days	-	(25)
		7 days	-	(25)
		2 weeks	-	(25)
		3 weeks	-	(25)
		4 weeks	-	(25)
		5 weeks	-	(25)
		6 weeks	-	(25)
		7 weeks	-	(25)
		8 weeks	-	(25)
		10 weeks	-	(25)
		12 weeks	-	(25)

mice	$1.7*10^{11}$ vp/kg	gonads	31 days	+	(93)
			91 days	+	(93)
dog	$2.0*10^{11}$ vp/kg	muscle, injected	12 weeks	+	(108)
human	$2*10^{11}$ vp/kg	muscle	2 months	+	(111)
			6 months	+	(111)
			12 months	+	(111)
		saliva	24 hours	+	(111)
			48 hours	-	(111)
			48 days	-	(111)
			56 days	-	(111)
			59 days	-	(111)
		semen	24 hours	-	(111)
			48 hours	-	(111)
			48 days	-	(111)
			56 days	-	(111)
			59 days	-	(111)
		serum	24 hours	+	(111)
			48 hours	+	(111)
			< 1 year	-	(111)
		stool	24 hours	-	(111)
			48 hours	-	(111)
			48 days	-	(111)
			56 days	-	(111)
			59 days	-	(111)
		urine	24 hours	+	(111)
			48 hours	-	(111)
			48 days	-	(111)
			56 days	-	(111)

			59 days	-	(111)
rat	$2.8*10^{11}$ vp/kg	epididymal effluent	15 days	+	(93)
dog	$1.2*10^{12}$ vp/kg	muscle, injected	12 weeks	+	(108)
mice	$1.7*10^{12}$ vp/kg	gonads	31 days	+	(93)
			91 days	+	(93)
dog	$2.0*10^{12}$ vp/kg	gonads	8 weeks	-	(107)
		kidney	8 weeks	-	(107)
		liver	8 weeks	-	(107)
		lung	8weeks	-	(107)
		muscle, contralateral	8 weeks	-	(107)
		muscle, perfused skeletal	8 weeks	+	(107)
		serum	1-5 days	+	(107)
			> 5 days	-	(107)
		spleen	8 weeks	-	(107)
dog	$4.0*10^{12}$ vp/kg	muscle, injected	12 weeks	+	(108)
monkey (Baboon)	$5.0*10^{12}$ vp/kg	gonads	4 months	-	(110)
rabbit	$1.0*10^{13}$ vp/kg	gonads	7 days	+	(93)
			30 days	+	(93)
			60 days	+	(93)
			90 days	+	(93)
		semen	7 days	-	(93)
			30 days	-	(93)
			60 days	-	(93)
			90 days	-	(93)

		serum	15 minutes	+	(93)
			24 hours	+	(93)
			48 hours	+	(93)
			7 days	-	(93)
rat	$2.8*10^{13}$ vp/kg	epididymal effluent	15 days	+	(93)
dog	$1.3*10^{11} – 1.1*10^{13}$ vp/kg	semen	1.5 months	-	(93)
			4 months	-	(93)
			14 months	-	(93)
			16 months	-	(93)
monkey (Rhesus and Cynomolgus)	$5.0*10^{8} – 1.0*10^{10}$ iu/kg	brain	8 to 18 months	-	(109)
		faeces	1 day	+	(109)
			2 days	+	(109)
			3 to 6 days	+	(109)
			7 days	-	(109)
		gonads	8 to 18 months	-	(109)
		heart	8 to 18 months	-	(109)
		intestine	8 to 18 months	-	(109)
		kidney	8 to 18 months	-	(109)
		lacrymal swab	6 hours	+	(109)
			1 day	+	(109)
			2 days	+	(109)
			3 to 6 days	+	(109)
			7 days	-	(109)
		liver	8 to 18 months	+	(109)

	lung	8 to 18 months	-	(109)
	lymph node	8 to 18 months	+	(109)
	muscle	8 to 18 months	+	(109)
	nasal swab	6 hours	+	(109)
		1 day	+	(109)
		2 days	+	(109)
		3 to 6 days	+	(109)
		7 days	-	(109)
	saliva	1 day	+	(109)
		2 days	+	(109)
		3 to 6 days	-	(109)
		7days	-	(109)
	serum	30 minutes	+	(109)
		6 hours	+	(109)
		1 day	+	(109)
		2 days	+	(109)
		3 to 6 days	+	(109)
		7 days	-	(109)
	spleen	8 to 18 months	-	(109)
	urine	6 hour	+	(109)
		1 day	+	(109)
		2 days	+	(109)
		3 to 6 days	+	(109)
		7 days	-	(109)

vp = vector particles
pfu = plaque forming units

iu = infectious units
MNC = mononucleated cells
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.19. Inhalatory and nasal-bronchial administration of AAV2

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
human	$3.0*10^{1} – 1.0*10^{9}$ ru	broncho-alveolar lavage	1 to 30 days	-	(115)
		nasal swab	1 to 30 days	-	(115)
		sputum	1 day	+	(115)
			7 to 30 days	-	(115)
		stool	1 to 30 days	-	(115)
		urine	1 to 30 days	-	(115)
human	$1.0*10^{2} – 1.0*10^{5}$ ru	blood	1 day	-	(114)
			7 days	-	(114)
		nose secretion	2 to 34 days	-	(114)
		stool	2 to 34 days	-	(114)
		urine	2 to 34 days	-	(114)
human	$1.0*10^{10} – 1.0*10^{13}$ drp	blood	1 day	+	(112)
		sputum	1 day	+	(112)
			7 days	+	(112)
			14 days	-	(112)
			30 days	-	(112)
		stool	1 to 90 days	-	(112)
		urine	1 to 90 days	-	(112)

human	$1.0*10^{13}$ drp	sputum	0 days	+	(113)
			14 days	+	(113)
			60 days	+	(113)
			75 days	+	(113)
			90 days	-	(113)
			150 days	+	(113)

ru = replicating units
drp = deoxyribonuclease-resistant particles
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.20. Ocular administration of AAV2

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
dog	$1.5*10^{8} – 4.5*10^{12}$ vp	chiasm	3 months	+	(116)
		diaphragm	3 months	+	(116)
		gonads	3 months	-	(116)
		heart	3 months	+	(116)
		jejunum	3 months	-	(116)
		kidney	3 months	-	(116)
		lateral geniculate nucleus	3 months	-	(116)
		liver	3 months	-	(116)
		lung	3 months	-	(116)
		lymph node, mandibular	3 months	+	(116)
		lymph node, parotid	3 months	-	(116)
		muscle, skeletal	3 months	-	(116)
		optic nerve, left	3 months	+	(116)
		optic nerve, right	3 months	-	(116)
		optic tract	3 months	-	(116)

		pancreas	3 months	-	(116)
		periocular	3 months	-	(116)
		spleen	3 months	-	(116)
		superior colliculus	3 months	-	(116)
		visual cortex	3 months	-	(116)
monkey (Cynomolgus)	$1.5*10^{12} – 3.3*10^{12}$ vp	cerebellum	1 week	-	(99)
		gonads	1 week	-	(99)
		heart	1 week	-	(99)
		jejunum	1 week	-	(99)
		kidney	1 week	-	(99)
		lateral geniculate nucleus, left	1 week	-	(99)
		lateral geniculate nucleus, right	1 week	+	(99)
		liver	1 week	-	(99)
		lung	1 week	-	(99)
		lymph node, mandibular	1 week	+	(99)
		lymph node, mesenteric	1 week	-	(99)
		lymph node, preauricular	1 week	+	(99)

lymph node, tracheobronchial	1 week	-	(99)
muscle, skeletal	1 week	-	(99)
optic chiasm	1 week	-	(99)
optic nerve, left	1 week	-	(99)
optic nerve, right	1 week	+	(99)
optic tract, left	1 week	+	(99)
optic tract, right	1 week	+	(99)
orbital tissue, left	1 week	-	(99)
orbital tissue, right	1 week	-	(99)
pancreas	1 week	-	(99)
retina, left	1 week	-	(99)
retina, right	1 week	+	(99)
spleen	1 week	-	(99)
superior colliculus, left	1 week	+	(99)
superior colliculus, right	1 week	-	(99)
thalamus	1 week	-	(99)

		visual cortex, left	1 week	+	(99)
		visual cortex, right	1 week	-	(99)
		vitreous, left	1 week	-	(99)
		vitreous, right	1 week	+	(99)
monkey (Cynomolgus)	$1.5*10^{12} – 3.3*10^{12}$ vp	cerebellum	3 months	-	(99)
		gonads	3 months	-	(99)
		heart	3 months	-	(99)
		jejunum	3 months	-	(99)
		kidney	3 months	-	(99)
		lateral geniculate nucleus, left	3 months	+	(99)
		lateral geniculate nucleus, right	3 months	-	(99)
		liver	3 months	-	(99)
		lung	3 months	-	(99)
		lymph node, mandibular	3 months	-	(99)
		lymph node, mesenteric	3 months	-	(99)
		lymph node, preauricular	3 months	-	(99)
		lymph node, tracheobronchial	3 months	-	(99)

	muscle, skeletal	3 months	-	(99)
	optic chiasm	3 months	-	(99)
	optic nerve, left	3 months	-	(99)
	optic nerve, right	3 months	-	(99)
	optic radiation, left	3 months	-	(99)
	optic radiation	3 months	-	(99)
	optic tract, left	3 months	-	(99)
	optic tract, right	3 months	-	(99)
	orbital tissue, left	3 months	-	(99)
	orbital tissue, right	3 months	-	(99)
	pancreas	3 months	-	(99)
	spleen	3 months	-	(99)
	superior colliculus, left	3 months	-	(99)
	superior colliculus, right	3 months	-	(99)
	thalamus	3 months	-	(99)
	visual cortex, left	3 months	-	(99)
	visual cortex, right	3 months	-	(99)

vp = vector particles
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.21. Salivary gland administration of AAV2

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
mice	$1*10^{9}$ vp	liver	8 weeks	+	(117)
		salivary glands	8 weeks	+	(117)
		spleen	8 weeks	+	(117)
		testes	8 weeks	+	(117)
monkey (Macaque)	$1*10^{10}$ vp	gonads	6 months	-	(118)
		heart	6 months	-	(118)
		kidney	6 months	-	(118)
		liver	6 months	-	(118)
		lung	6 months	-	(118)
		lymph node, cervical	6 months	-	(118)
		parotid gland, left	6 months	-	(118)
		parotid gland, right	6 months	+	(118)
		spleen	6 months	-	(118)
		submandibular gland	6 months	-	(118)

monkey (Macaque)	$1*10^{11}$ vp	gonads	6 months	-	(118)
		heart	6 months	-	(118)
		kidney	6 months	-	(118)
		liver	6 months	-	(118)
		lung	6 months	-	(118)
		lymph node, cervical	6 months	-	(118)
		parotid gland, left	6 months	-	(118)
		parotid gland, right	6 months	+	(118)
		spleen	6 months	-	(118)
		submandibular gland	6 months	-	(118)

vp = vector particles
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.22. Intra-cochlear and intra-articular administration of AAV2

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
guinea pigs	$5*10^8$ vp	cerebellum	2 weeks	+	(120)
			4 weeks	+	(120)
		cochlea, control	2 weeks	+	(120)
			4 weeks	+	(120)
		cochlea, injected	2 weeks	+	(120)
			4 weeks	+	(120)
		cortex	2 weeks	-	(120)
			4 weeks	-	(120)
		heart	2 weeks	-	(120)
			4 weeks	-	(120)
		kidney	2 weeks	-	(120)
			4 weeks	-	(120)
		liver	2 weeks	-	(120)
			4 weeks	-	(120)
		lung	2 weeks	-	(120)
			4 weeks	-	(120)
rabbit	$1.5*10^{12}$ iu	serum	21 days	-	(119)

vp = vector particles
iu = infecrtious units
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.23. Intra-cerebral lateral ventricle administration of AAV2

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
mice	$1.8*10^{10}$ vp	brain, fore caudal	30 minutes	+	(121)
			20 hours	+	(121)
		brain, fore rostal	30 minutes	+	(121)
			20 hours	+	(121)
		brain, hind	30 minutes	+	(121)
			20 hours	+	(121)
		brain, mid	30 minutes	+	(121)
			20 hours	+	(121)
		brain, parenchyma	1 week	+	(121)
		meninges	1 week	+	(121)
		pial surface	1 week	+	(121)

vp = vector particles
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Table A.24. Isolated limb perfusion administration of AAV2

Species	Dose (vp, pfu or iu)	Organ/Excreta	Time	+/-	References
dog	$2*10^{12}$ vp/kg	kidney	8 weeks	-	(107)
		liver	8 weeks	-	(107)
		lung	8 weeks	-	(107)
		muscle, control	8 weeks	-	(107)
		muscle, perfused	8 weeks	+	(107)
		ovary	8 weeks	-	(107)
		serum	1 to 5 days	+	(107)
			> 5 days	-	(107)
		spleen	8 weeks	-	(107)

vp = vector particles
- = not detected using the detection method described in the reference
+ = detected using the detection method described in the reference

Appendix B. Detailed biodistribution and shedding tables of Ad and AAV (CD-ROM)

The detailed information on the biodistribution, shedding and analysis techniques for the different adenoviral and adeno-associated viral vector studies are provided on a CD-Rom.

Appendix B. Detailed biodistribution and shedding tables of Ad and AAV

The detailed information on the biodistribution, shedding and analysis techniques for the different adenoviral and adeno-associated viral vector studies are provided on this CD-Rom.

Table B.1. Intravenous and intra-arterial administration of Ad

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	results	analysis technique	time point	tested compartments	results	analysis technique		
Stone et al., 2007	Ad3	mice (hCD46)	?	intra-venous (tail vein)	$2x10^{10}$ vp	6 h	6 h	liver lung spleen	2.5 0.6 2.5	Q-PCR						data based on figure
Stone et al., 2007	Ad3	mice (hCD46)	?	intra-venous (tail vein)	$1x10^{11}$ vp	6 h	10 min	blood cells serum	0.1 0.1	Q-PCR						data based on figure
							6 h	liver lung spleen	6 2 4							
Stone et al., 2007	Ad4	mice (hCD46)	?	intra-venous (tail vein)	$2x10^{10}$ vp	6 h	6 h	liver lung spleen	0.6 0.06 0.3	Q-PCR						data based on figure
Stone et al., 2007	Ad4	mice (hCD46)	?	intra-venous (tail vein)	$1x10^{11}$ vp	10 min	10 min	blood cells serum	2.8 2.3	Q-PCR						data based on figure
Stone et al., 2007	Ad5	mice (hCD46)	?	intra-venous (tail vein)	$1x10^{11}$ vp	10 min	10 min	blood cells serum	0.5 0.4	Q-PCR						data based on figure
								liver lung spleen	11 0.6 7.5							
Johnson et al., 2006	Ad5	mice (SCID)	male	intra-venous	$1x10^{8}$ pfu	14 days	14 days	lung liver kidney spleen prostate	1.5% 100% 2% 42.4% 0.1%	RT-PCR						
								lung liver kidney spleen prostate	100% 0.8% 0.7% 0.2% 0.6%	in vivo optical imaging						
									expressed relative to liver							
Tolcher et al., 2006	Ad5	human (patients with advanced malignancies)	male and female (n = 17)	intra-venous (on 1 day, 2, 3 and every 28 days)	$3x10^{10}$ – $3x10^{12}$ vp	28 days	1 day 2 days 5 days 14 days 28 days	plasma	+ (10/10) + (8/10) + (4/10) + (5/10) + (2/8)		samples collected during course	urine	1/74 obtained from 10 patients	PCR		
											samples collected during course	rectal swab	1/53 obtained from 6 patients			
											samples collected during course	oral rinse	0 obtained from 15 patients			

Tolcher et al., 2006	Ad5	human (patients with advanced malignancies)	male and female (n = 17)	intra-venous (on 1 day, 2, 3 and every 28 days)	$3x10^{10}$ vp	28 days	1 day 2 days 5 days 14 days 28 days	plasma	+ (1/1) - (0/1) - (0/1) - (0/1) - (0/1)	
Tolcher et al., 2006	Ad5	human (patients with advanced malignancies)	male and female (n = 17)	intra-venous (on 1 day, 2, 3 and every 28 days)	$1x10^{11}$ vp	28 days	1 day 2 days 5 days 14 days 28 days	plasma	+ (3/3) + (3/3) - (0/3) - (0/3) - (0/2)	
Tolcher et al., 2006	Ad5	human (patients with advanced malignancies)	male and female (n = 17)	intra-venous (on 1 day, 2, 3 and every 28 days)	$3x10^{11}$ vp	28 days	1 day 2 days 5 days 14 days	plasma	+ (1/1) - (0/1) + (1/1) + (1/1)	
Tolcher et al., 2006	Ad5	human (patients with advanced malignancies)	male and female (n = 17)	intra-venous (on 1 day, 2, 3 and every 28 days)	$1x10^{12}$ vp	28 days	1 day 2 days 5 days 14 days 28 days 4 days	plasma tumour biopsy	+ (5/5) + (5/5) + (3/5) + (4/5) + (2/3) +	
Tolcher et al., 2006	Ad5	human (patients with advanced malignancies)	male and female (n = 17)	intra-venous (on 1 day, 2, 3 and every 28 days)	$1x10^{12}$ vp	28 days	4 days	tumour biopsy	+	
Ni et al., 2005	Ad5	monkey (Baboon)	male (n=6)	intra-venous (femoral vein)	$2x10^{12}$ vp/kg	3 days	72 h	liver spleen lung kidney brain heart pancreas oesophagus stomach duodenum testis inguinal lymph nodes mesenteric lymph nodes axillary lymph nodes bone marrow MNC bone marrow MNC peripheral blood leukocytes	$6x10^{4}$ $1x10^{4}$ $5x10^{3}$ $3x10^{3}$ $9x10^{3}$ $3x10^{3}$ $5x10^{2}$ $6x10^{2}$ $1x10^{4}$ $9x10^{2}$ $8x10^{1}$ $9x10^{2}$ $1x10^{3}$ $5x10^{2}$ $3x10^{2}$ 40 0	Q-PCR (viral genomes/ 10000 cells) β-galactosidase expression
Wang et al., 2004b	Ad5 (gutless)	mice (C57BL/6J)	?	intra-venous (tail vein)	$3x10^{9}$ iu	2 weeks, 28 weeks and 49 weeks	liver lung spleen kidney	++ + + +	PCR	

Wen et al., 2003	Ad5 (rAd.p21)	rabbit (New Zealand White)	female	intra-venous (n=1)	$9.5x10^{11}$ vp	5 min 30 min 2 h 24 h	blood	$1.8x10^{8}$ $1.3x10^{7}$ $1.8x10^{6}$ $1x10^{6}$	Q-PCR (GE/ml)	
						1 day	spleen liver lungs heart ovaries kidney eye	$1.3x10^{6}$ $1.7x10^{5}$ $1.4x10^{4}$ $1.3x10^{3}$ $1.5x10^{4}$ 0 $1.4x10^{3}$	Q-PCR (GE/mg)	
							spleen liver lungs heart ovaries kidney eye	$1.2x10^{6}$ $1.7x10^{6}$ $1.4x10^{4}$ $1x10^{3}$ $1.9x10^{4}$ $1.7x10^{3}$ $1.2x10^{3}$	Q-RT-PCR (copies/mg)	
Turunen et al., 2002	Ad5 (control)	rabbit (New Zealand White)	? (n=22)	intra-arterial (renal artery)	$1.15x10^{10}$ pfu	2 weeks	aorta	+	RT-PCR	
							liver	+	X-gal staining	
							liver	+ (control level 4x targeted)	Q-RT-PCR	
Alemany et al., 2001	Ad5 (control) and Ad mutated (CAR-binding ablated)	mice (C57BLl/6)	female	intra-venous (tail vein)	$5x10^{10}$ vp	90 min	lung liver kidney liver kidney	$5x10^{3}$-$1x10^{4}$ $1x10^{3}$-$4x10^{3}$ $6.3x10^{4}$-$7.6x10^{4}$ Abl > control Abl > control	Q-PCR (copies/ ng DNA) luciferase activity	
Kosuga et al 2000	Ad5	mice		intravenous (tail vein)	$1x10^{8}$ pfu	7 days	liver spleen kidney lung heart	+++ +++ + + ++	transgene expression	
Alemany et al., 2000	Ad5	mice (BALB/c)	? (n = 3)	intra-venous (vena cava)	$1x10^{10}$ tu	0-1 h	blood	$t_0 = 5x10^{6}$ tu/µl 1-5 min 7-fold reduction in virus titre from $6.5x10^{5}$ to $1x10^{5}$ tu/µl after 1 h $5x10^{2}$ tu/µl	infection of A549 cells	$t_{½} < 2$ min $K_{1\text{-}5min} = 0.2$ $K_{1\text{-}30min} = 0.08$ (K = clearance rate constant)
Hackett et al., 2000	Ad5	pig (Yorkshire)	male	intra-venous (peripheral vein)	10^{12} vp	24 h	lung liver spleen left ventricle	90 8 2 0	Q-PCR (%of total recovered)	

Hackett et al., 2000	Ad5	pig (Yorkshire)	male	intra-venous (hepatic portal vein)	10^{12} vp		24 h	lung liver spleen left ventricle	56 43 1 0	Q-PCR (%of total recovered)					
Hiltunen et al., 2000	Ad5	rabbit (New Zealand White)	male	intra-arterial (carotid artery)	$1.15x10^{10}$ pfu (n=3)	14 days	14 days	target vessel control vessel white blood cells bone marrow skeletal muscle testis epididymis liver heart lung cerebra cerebellum spleen kidney	1.1 0 1.8 <0.01 0 0.06 <0.01 0.7 0 <0.01 0 0 <0.01 <0.01	X-gal staining (%)	14 days	sperm	0 +	X-gal staining (%) RT-PCR
								target vessel control vessel white blood cells bone marrow skeletal muscle testis epididymis liver heart lung cerebra cerebellum spleen kidney	+ - + + + + - + + + - - + +	RT-PCR				
Hiltunen et al., 2000	Ad5	rabbit (New Zealand White)	male	peri-adventitial (carotid artery)	$1.15x10^{10}$ pfu (n=3)	14 days	14 days	target vessel control vessel white blood cells bone marrow skeletal muscle testis epididymis liver heart lung cerebra cerebellum spleen lymph node kidney	0.1 0 0 <0.01 <0.01 <0.01 0 <0.01 0 0 0 0 <0.01 0.05 0	X-gal staining (%)	14 days	sperm	0 +	X-gal staining (%) RT-PCR
								target vessel control vessel bone marrow skeletal muscle testis epididymis liver	+ - + + + - +	RT-PCR				

								heart	-	
								lung	+	
								cerebra	-	
								cerebellum	-	
								spleen	-	
								lymph node	+	
								kidney	-	
Wood et al., 1999	Ad5	mice (BALB/c)	female	intra-venous (tail vein)	$5x10^9$ pfu	72 h	72 h	bladder	6-30	luciferase assay (RLU/mg)
								liver	$9x10^4$-$3x10^5$	
								kidney	15-30	
								heart	150	
								ureter	10-100	
								lung	70-200	
								bladder	250	PCR (vector copies)
								liver	≥2500	
								kidney	≥2500	
								heart	≥2500	
								ureter	≥2500	
								adrenal	250	
								lung	≥ 2500	
Cichon et al., 1999	Ad5 (E1 deleted)	rabbit (New Zealand White)		intra-venous (ear vein)	$5x10^{11}$ inf particles	60 days	60 days	liver	+ (50-70% hepatocytes)	X-gal staining
Cichon et al., 1999	Ad5 (E1 deleted)	rabbit (New Zealand White)		intra-venous (portal vein)	$5x10^{10}$ inf particles	60 days	10 min	plasma	10^8	titration (inf particles/ml)
							30 min		10^8	
							60 min		10^7	
							4 h		10^6	
							24 h		10^4	
							48 h		10^2	
Cichon et al., 1999	Ad5 (E1 deleted)	rabbit (New Zealand White)		intra-venous (portal vein)	$5x10^{11}$ inf particles	60 days	10 min	plasma	10^8	titration (inf particles/ml)
							30 min		10^9	
							60 min		10^8	
							4 h		10^8	
							24 h		10^6	
							48 h		10^2	
Kuzmin et al., 1997	Ad5	mice (BALB/c)	male	intra-venous (tail vein)	10^9 pfu	6 weeks	10 min	bile	10^5-10^6 vp/µg	PCR
							90 min		10^5-10^6 vp/µg	
							8 h		10^5 vp/µg	
							24 h		10^4-10^5 vp/µg	
							10 min	liver	10^5-10^6 vp/µg	
							90 min		10^5-10^6 vp/µg	
							8 h		10^5 vp/µg	
							24 h		10^4-10^5 vp/µg	
							10 min	liver	10-100 vp/cell	
							30 min		10-100 vp/cell	
							90 min		10-100 vp/cell	
							3 h		10-100 vp/cell	
							8 h		10 vp/cell	
							24 h		1-10 vp/cell	
							1 week		1-10 vp/cell	
							2 weeks		1-10 vp/cell	
							6 weeks		0.1 – 1 vp /cell	

Stone et al., 2007	Ad5/11	mice (hCD46)	?	intra-venous (tail vein)	$1x10^{11}$ vp	10 min	10 min	blood cells serum	0.4 0.5	Q-PCR	data based on figure
Ni et al., 2005	Ad5/11	monkey (Baboon)	male (n=6)	intra-venous (femoral vein)	$2x10^{12}$ vp/kg	3 days	72 h	liver spleen lung kidney brain heart pancreas oesophagus stomach duodenum testis inguinal lymph nodes mesenteric lymph nodes axillary lymph nodes bone marrow BM-MNC peripheral blood leukocytes	$1.5x10^{3}$ $1.3x10^{4}$ $1.2x10^{3}$ $1.7x10^{2}$ $1.4x10^{2}$ $1.4x10^{3}$ $1.6x10^{2}$ $1.7x10^{2}$ $1.4x10^{2}$ $1.7x10^{1}$ $1.1x10^{1}$ $1.4x10^{1}$ $1.1x10^{1}$ $1.3x10^{1}$ $1x10^{2}$ 255 0.1	Q-PCR (viral genomes/ 10000 cells)	

Stone et al., 2007	Ad5/35	mice (hCD46)	?	intra-venous (tail vein)	$1x10^{11}$ vp	10 min	10 min	blood cells serum	0.2 0.3	Q-PCR	data based on figure
Ni et al., 2005	Ad5/35	monkey (Baboon)	male (n=6)	intra-venous (femoral vein)	$2x10^{12}$ vp/kg	3 days	72 h	liver spleen lung kidney brain heart pancreas oesophagus stomach duodenum testis inguinal lymph nodes mesenteric lymph nodes axillary lymph nodes bone marrow BM-MNC peripheral blood leukocytes	$1.7x10^{3}$ $1.7x10^{4}$ $1.3x10^{3}$ $1.8x10^{2}$ $1.7x10^{2}$ $1.5x10^{3}$ $1.5x10^{2}$ $1.8x10^{2}$ $1.6x10^{2}$ $1.8x10^{1}$ $1.2x10^{1}$ $1.8x10^{1}$ $1.3x10^{1}$ $1x10^{1}$ $1.8x10^{1}$ 684 1	Q-PCR (viral genomes/ 10000 cells)	
							30 days	liver spleen lung kidney brain heart pancreas oesophagus stomach duodenum prostate	$1.4x10^{3}$ $1.2x10^{3}$ $1.8x10^{2}$ $1.6x10^{1}$ $1.8x10^{1}$ $1.7x10^{2}$ $1.5x10^{1}$ $1.6x10^{1}$ $1.3x10^{1}$ $0.6x10^{1}$ $1.8x10^{1}$		

								testis	$0.6x10^1$		
								inguinal lymph nodes	$0.8x10^1$		
								mesenteric lymph nodes	$0.5x10^1$		
								axillary lymph nodes	$1.3x10^1$		
							1 min	blood cells	$1.5x10^3$		
							30 min		$1.6x10^3$		
							6 h		$1.2x10^4$		
							24 h		$1.9x10^3$		
							72 h		$1x10^3$		
							8 days		-		
							10 days		-		
							14 days		-		
							17 days		-		
							21 days		-		
							24 days		-		
Turunen et al., 2002	Ad5/? (targeted)	rabbit (New Zealand White)	? (n=22)	intra-arterial (renal artery)	$1.15x10^{10}$ PFU		2 weeks	aorta	+	RT-PCR	modified surface
								liver	-	X-gal staining	
								liver	+	Q-RT-PCR	
Stone et al., 2007	Ad11	mice (hCD46)	?	intra-venous (tail vein)	$1x10^{11}$ vp	10 min	10 min	blood cells	1.4	Q-PCR	data based on figure
								serum	1.9		
								liver	7		
								lung	0.6		
								spleen	2		
Stone et al., 2007	Ad35	mice (hCD46)	?	intra-venous (tail vein)	$1x10^{11}$ vp	10 min	10 min	blood cells	0.01	Q-PCR	data based on figure
								serum	0.01		
								liver	9		
								lung	8		
								spleen	20		
Stone et al., 2007	Ad41	mice (hCD46)	?	intra-venous (tail vein)	$1x10^{11}$ vp	10 min	10 min	blood cells	0.01	Q-PCR	data based on figure
								serum	0.02		
								liver	2		
								lung	0.3		
								spleen	3.5		

Table B.2. Intra-peritoneal and intra-pleural administration of Ad

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	results	analysis technique	time point	tested compartments	results	analysis technique		
Johnson et al., 2006	Ad5	mice (SCID)	male	intra-peritoneal	$1x10^8$ pfu	14 days	14 days	lung liver kidney spleen prostate	0.1% 100% 12.9% 7.8% 10.4%	RT-PCR						
								lung liver kidney spleen prostate	1.3% 100% 20.7% 10.2% 2.0%	in vivo optical imaging						
									expressed relative to liver							
Wolf et al., 2004	Ad5	human (patients with ovarian cancer)	female (n = 15)	intra-peritoneal	$3x10^{10}$ – $3x10^{12}$ vp (5 days every 3 weeks)		1-6 days, 8 days, 15 days and 22 days	ascites/peritoneal washings	-	viral cytopathic effect	1-6 days, 8 days, 15 days and 22 days	urine stool sputum	- - -	viral cytopathic effect		
Buller et al., 2002	Ad5	human (patients with recurrent ovarian cancer)	female (n = 41)	intra-peritoneal	$7.5x10^{10}$-$7.5x10^{13}$ vp		72 h	pleural fluid (n=1)	+	ELISA	during treatment and before discharge	urine stool	- -	ELISA		
							6-7 days until >1year	peritoneal fluid	+	ELISA						
							6-7 days until >1year	peritoneal fluid	-	culture or infection						
							6-7 days until >1year	ascitic fluid	+	PCR						
							6-7 days until >1year	laparoscopic biopsy	+							
Sterman et al., 2000	Ad5	human (patients with malignant pleural mesothelioma	male and female) (n = 10)	into the pleural cavity	$1.5x10^{13}$ vp	30 days	1 day	blood	-	PCR	1 day	nasal swab	-	PCR		
							3 days		-		3 days		-			
							5 days		-		5 days		-			
							7 days		-		7 days		-			
							14 days		-		14 days		-			
							19 days		-		19 days		-			
											1 day	urethral swab	-			
											3 days		-			
											5 days		-			
											7 days		-			
											14 days		-			
											19 days		-			
											1 day	rectal swab	-			
											3 days		-			
											5 days		-			
											7 days		-			
											14 days		-			
											19 days		-			

Table B.3. Intra-tumoral administration of Ad

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	results	analysis technique	time point	tested compartments	results	analysis technique		
Sauthoff et al., 2003	Ad309 (wild type)	mice (NCrNU-M)	?	intra-tumoral	$1x10^9$ pfu	4 weeks	4 weeks	tumour lung liver	virus is located preferentially in the vicinity of connective tissue and in proximity to intra-tumoral blood vessels	immuno-histochemistry						
Fujiwara et al., 2006	Ad5	human (NSCLC patients)	male and female (n = 12)	intra-tumoral (lung tumour)	$10^9 – 10^{11}$ pfu	?	30 min	plasma	+	PCR	1 day	gargle	92%	PCR (of all patients)		
											2 days		67%			
							25 and 151 days (tested for two patients that died during trial)	tumour	+		3 days		42%			
								proximal lymph node	+		4 days		33%			
								liver	-		5 days		42%			
								kidney	-		6 days		33%			
								testis	-		7 days		17%			
								distal lymph nodes	-		8 days		17%			
											9 days		33%			
											10 days		17%			
											11 days		17%			
											12 days		17%			
											13 days		17%			
											14 days		8%			
											15 days		8%			
											1 day	urine	0 %			
											2 days		0 %			
											3 days		8%			
											4 days		8%			
											5 days		17%			
											6 days		8%			
											7 days		8%			
											8 days		0 %			
											9 days		8%			
											10 days		8%			
											11 days		8%			
											12 days		8%			
											13 days		8%			
											14 days		8%			
											15 days		0 %			

Cunningham et al., 2005	Ad5	human (patients with resectable solid tumours)	male and female (n = 28)	intra-tumoral	$2x10^{10}$ – $2x10^{12}$ vp	?	1-4 days tumour resection	injection site	$<1x10^{8}$ copies at centre injection site	PCR				
									1 cm from injection sites DNA copies reduced by 90%					
							1 day		$1x10^{8}$ vector DNA/µg					
							2 days		$1x10^{8}$ vector DNA/µg					
							4 days		$1x10^{5}$ vector DNA/µg					
							30 days		$3x10^{3}$ vector DNA/µg					
Tong et al., 2005	Ad5	human (patients with advanced cancer)	male and female (n = 22)	intra-tumoral	$2x10^{10}$ – $2x10^{12}$ vp		30 min	plasma	3% of injected vp	PCR				vector half-life 11 min
							24 h		0.0025% of injected vp					
Wang et al., 2005a	Ad5	mice (C57BL/6, BALB/c and nude BALB/c)	female	intra-tumoral	$2x10^{8}$ pfu		24h	liver tumour	+ +	luciferase activity				peak value 10 min plasma half-life Ad is ~ 2 min
Wang et al., 2005a	Ad5	mice (C57BL/6, BALB/c and nude BALB/c)	female	intra-tumoral	$3x10^{8}$ pfu		25 sec 50 sec 5 min 10 min 2h, 24h	blood	+ + + + (max) +/- -	qualitative infectious units assay				peak value 10 min plasma half-life Ad is ~ 2 min
							10 min	liver tumour	liver/tumour virus copy nr ratio 0.2-15.5	Q-PCR				
Mundt et al., 2004	Ad5	human (patients with soft tissue carcinoma)	male and female (n = 13)	intra-tumoral	$4x10^{9}$ – $4x10^{11}$ vp		2 weeks after completion of treatment	blood	-	PCR	2 weeks	sputum urine	- -	PCR
Palmer et al., 2004	Ad5	human (patients with (colorectal) liver cancer)	male and female (n = 18)	intra-tumoral	$1x10^{8}$ – $5x10^{11}$ vp		24 h	plasma	-	ELISA	24 h	throat swab urine stool	- - -	ELISA
							30 min	blood	+	PCR				
							1 h 2 h 4 h 8 h		declining compared to 30 min					
							24 h		+ in 2/13					

Germano et al., 2003	Ad5	human (patients with glioblastoma)	male and female (n = 11)	intra-tumoral (brain)	$2.5x10^{11}$ - $5.6x10^{12}$ vp	248 weeks	24 h	blood	-	IF techniques PCR	24 h	nasal swab urine	- -	IF techniques PCR
Griscelli et al., 2003	Ad5	human (patients with NSCLC)	male and female (n = 21)	intra-tumoral (bronchial)	$1x10^{7}$ pfu	90 days	0 days	PBL	16% 0%	PCR cell culture	0 days	bronchial	100% 100%	PCR cell culture
							0 days 2 days 7 days	plasma	66% 66% 33%	PCR	2 days 4-5 days 11 days	sputum	33% 50% 16%	PCR
							0 days	plasma	0%	cell culture	2 days	sputum	0%	cell culture
											2 days	throat swabs faeces urine	0% 0% 0%	PCR
												throat swabs faeces urine	0% 0% 0%	cell culture
											8 days 15 days 30 days 60 days	bronchial	100% 66% 25% 0%	PCR
Griscelli et al., 2003	Ad5	human (patients with NSCLC)	male and female (n = 21)	intra-tumoral (bronchial)	$1x10^{8}$ pfu	90 days	0 days	PBL	100% 0%	PCR cell culture	0 days	bronchial	100% 100%	PCR cell culture
							0 days 2 days	plasma	100% 0%	PCR	2 days 13 days	sputum	33% 16%	PCR
							0 days	plasma	0%	cell culture	2 days	sputum	0%	cell culture
											2 days	throat swabs faeces urine	0% 16% 0%	PCR
											2 days	throat swabs faeces urine	0% 0% 0%	cell culture
											8 days 15 days 30 days 60 days	bronchial	83% 66% 60% 0%	PCR
											9-11 days	throat swabs	16%	PCR

Griscelli et al., 2003	Ad5	human (patients with NSCLC)	male and female (n = 21)	intra-tumoral (bronchial)	$1x10^9$ pfu	90 days	0 days 2 days 4 days	PBL	100% 44% 22%	PCR	0 days 8-10 days 12-15days 30 days	bronchial	100% 100% 100% 100%	PCR
							0 days 2 days	PBL	44% 0%	cell culture PCR	60 days 90 days		83% 75%	
							0 days 2 days 4 days	plasma	100% 33% 33%		0 days	bronchial	100%	cell culture
							0 days 2 days	plasma	33% 0%	cell culture	2 days 3-4 days 8-10 days 12-15days 30 days 60 days 90 days	sputum	62% 56% 62% 33% 22% 12% 0%	PCR
											2 days	throat swabs faeces urine	33% 33% 0%	PCR
											2 days	sputum throat swabs faeces urine	22% 0% 0% 0%	cell culture
Trask et al., 2000	Ad5	human (patients with advanced recurrent malignant brain tumours)	male and female (n = 13)	intra-tumoral	$2x10^9$ - $2x10^{12}$ vp	2 years	1 week 2 weeks 4 weeks	serum	- - -	plaque forming assay	hospitalisation 2 weeks	urine	- - -	PCR
											1 week 2 weeks 4 weeks	urine	- - -	plaque forming assay
											1 week 2 weeks 4 weeks	nasal swabs	- - -	plaque forming assay
Nemunaitis et al., 2000	Ad5	human (patients with advanced NSCLC)	male and female (n = 24)	intra-tumoral	$1x10^6$ pfu						0-24 h	urine sputum saliva	+ + +	CPE assay
											9 days	urine sputum saliva	- - -	
Nemunaitis et al., 2000	Ad5	human (patients with advanced NSCLC)	male and female (n = 24)	intra-tumoral	$1x10^8$ pfu		30 min 60 min 90 min	plasma	+ + +	PCR	0-24 h	urine sputum saliva	+ + +	CPE assay
							30 min 60 min 90 min		$1x10^6$ $1x10^6$ $1x10^5$	CPE assay	9 days	urine sputum saliva	- - -	

Nemunaitis et al., 2000	Ad5	human (patients with advanced NSCLC)	male and female (n = 24)	intra-tumoral	1x10^{9} pfu	30 min 60 min 90 min	plasma	+ + -/+	PCR	0-24 h	urine sputum saliva	+ + +	CPE assay
						30 min 60 min 90 min		10^{4}-10^{6} 10^{5}-10^{6} 10^{3}-10^{5}	CPE assay	9 days	urine sputum saliva	- - -	
Nemunaitis et al., 2000	Ad5	human (patients with advanced NSCLC)	male and female (n = 24)	intra-tumoral	3x10^{9} pfu	30 min 60 min 90 min	plasma	-/+ + -/+	PCR	0-24 h	urine sputum saliva	+ + +	CPE assay
						30 min 60 min 90 min		10^{3}-10^{6} 10^{5} 10^{3}-10^{6}	CPE assay	9 days	urine sputum saliva	- - -	
Nemunaitis et al., 2000	Ad5	human (patients with advanced NSCLC)	male and female (n = 24)	intra-tumoral	1x10^{10} pfu	30 min 60 min 90 min	plasma	+ + +	PCR	0-24 h	urine sputum saliva	+ + +	CPE assay
						30 min 60 min 90 min		10^{6} 10^{4}-10^{5} 10^{2}-10^{3}	CPE assay	9 days	urine sputum saliva	- - -	
Nemunaitis et al., 2000	Ad5	human (patients with advanced NSCLC)	male and female (n = 24)	intra-tumoral	3x10^{10} pfu	30 min 60 min 90 min	plasma	+ + +	PCR	0-24 h	urine sputum saliva	+ + +	CPE assay
						30 min 60 min 90 min		10^{6}-10^{7} 10^{5} 10^{3}	CPE assay	9 days	urine sputum saliva	- - -	
Nemunaitis et al., 2000	Ad5	human (patients with advanced NSCLC)	male and female (n = 24)	intra-tumoral	1x10^{11} pfu	30 min 60 min 90 min	plasma	+/- +/- -/+	PCR	0-24 h	urine sputum saliva	+ + +	CPE assay
						30 min 60 min 90 min		10^{5}-10^{7} 10^{3}-10^{6} 10^{1}-10^{5}	CPE assay	9 days	urine sputum saliva	- - -	
Stewart et al., 1999	Ad5	human (breast cancer (n=8) or melanoma (n=15) patients)		intra-tumoral	1x10^{7} - 1x10^{10} pfu	24 h	blood	+ (1/23)	Southern blot	24 h	urine stool throat swab	- - -	PCR or bio-assay for replication competent Ad
						24 h	blood	-	PCR for replication competent Ad	7 days	urine stool throat swab	- - -	
						7 days	tumour biopsy	-	PCR for replication competent Ad	24 h	urine stool throat swab	- - -	Southern blot
						7 days	tumour biopsy	+ (18/22)	PCR for vector DNA	7 days	urine stool throat swab	- - -	

Clayman et al., 1998	Ad5	human (patients with recurrent or refractory squamous cell carcinoma)	male and female (n=34)	intra-tumoral	$1x10^6$ - $1x10^{11}$ pfu (6 doses/ course, 1-7 courses)	30 min 48 h 30 min 90 min 24 h 48 h	blood	+ - +(>$3x10^{10}$) +(>$3x10^{10}$) +(>$3x10^{10}$) -	PCR CPE assay	1-7 days > 7 days 1-7 days > 7 days 1 day 3-17days > 17days	saliva sputum urine	+ - + - + + -	CPE assay	serum and urine of health care providers were negative (PCR and CPE assay)
Tursz et al., 1996	Ad5	human (lung cancer patients)	male and female	intra-tumoral	10^7 or 10^8 pfu	1 day 8 days 1 month 2 months 3 months 8 days 1 month 2 months 3 months	blood tumour biopsy tumour biopsy	+ (3/6) + (2/6) + (1/6) - - + (5/6) + (1/6) + (2/6) + (2/4)	PCR β-gal expression PCR	5-13 days	sputum	+ (3/6)	PCR	hospital staff (throat and stool samples) remained - for recombinant Ad infection

Table B.4. Dermal administration of Ad

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	results	analysis technique	time point	tested compartments	results	analysis technique		
Plog et al., 2006	Ad2	mice (C57BL/6)	male and female (n = 20) (n = 5 per time point)	intra-dermal (near each scapula and hip) (4 injection places)	$5x10^8$ vp (single dose)	92 days	2 days	blood	-	RT-PCR, (LLOQ 1 copy/µg DNA)						
							14 days		-							
							2 days	bone marrow	10%							
							14 days		-							
							28 days		-							
							2 days	brain	-							
							14 days		-							
							2 days	gonads	-							
							14 days		-							
							2 days	heart	-							
							14 days		-							
							2 days	injection site	100%							
							14 days		60%							
							28 days		30%							
							91 days		50%							
							2 days	kidney	-							
							14 days		-							
							2 days	liver	-							
							14 days		-							
							2 days	lung	-							
							14 days		-							
							2 days	lymph nodes	80%							
							14 days		40%							
							28 days		70%							
							91 days		10%							
							2 days	spleen	-							
							14 days		-							
Plog et al., 2006	Ad2	mice (C57BL/6)	male and female (n = 20) (n = 5 per time point)	intra-dermal (near each scapula and hip) (4 injection places)	$5x10^9$ vp (single dose)	92 days	2 days	blood	-	RT-PCR, (LLOQ 1 copy/µg DNA)						
							14 days		-							
							2 days	bone marrow	-							
							14 days		-							
							2 days	brain	-							
							14 days		-							
							2 days	gonads	-							
							14 days		-							
							2 days	heart	-							
							14 days		-							

							2 days	injection site	100%	
							14 days		30%	
							28 days		60%	
							91 days		60%	
							2 days	kidney	-	
							14 days		-	
							2 days	liver	-	
							14 days		-	
							2 days	lung	10%	
							14 days		-	
							28 days		-	
							2 days	lymph nodes	100%	
							14 days		80%	
							28 days		80%	
							91 days		40%	
							2 days	spleen	-	
							14 days		-	
Plog et al., 2006	Ad2	mice (C57BL/6)	male and female (n = 20) (n = 5 per time point)	intra-dermal (near each scapula and hip) (4 injection places)	$5x10^8$ vp (6 weekly doses)	126 days	38 days	blood	-	RT-PCR, (LLOQ 1 copy/µg DNA)
							50 days		-	
							38 days	bone marrow	-	
							50 days		-	
							38 days	brain	-	
							50 days		-	
							38 days	gonads	-	
							50 days		-	
							38 days	heart	-	
							50 days		-	
							38 days	injection site	100%	
							50 days		100%	
							64 days		100%	
							126 days		30%	
							38 days	kidney	-	
							50 days		-	
							38 days	liver	-	
							50 days		-	
							38 days	lung	-	
							50 days		-	
							38 days	lymph nodes	89%	
							50 days		60%	
							64 days		40%	
							126 days		10%	

							38 days	spleen	22%	
							50 days		20%	
							64 days		-	
							126 days		-	
Plog et al., 2006	Ad2	mice (C57BL/6)	male and female (n = 20) (n = 5 per time point)	intra-dermal (near each scapula and hip) (4 injection places)	$5x10^9$ vp (6 weekly doses)	126 days	38 days	blood	11%	RT-PCR, (LLOQ 1 copy/µg DNA)
							50 days		-	
							64 days		-	
							38 days	bone marrow	-	
							50 days		-	
							38 days	brain	-	
							50 days		-	
							38 days	gonads	-	
							50 days		-	
							38 days	heart	-	
							50 days		-	
							38 days	injection site	100%	
							50 days		100%	
							64 days		100%	
							126 days		90%	
							38 days	kidney	-	
							50 days		-	
							38 days	liver	-	
							50 days		-	
							38 days	lung	-	
							50 days		-	
							38 days	lymph nodes	89%	
							50 days		60%	
							64 days		80%	
							126 days		10%	
							38 days	spleen	56%	
							50 days		30%	
							64 days		40%	
							126 days		-	

Gu et al., 2004	Ad5	rabbit (New Zealand White)	?	dermal (wound application)	$7x10^{10}$ vp (4 weekly doses)	22 days	22 days	blood	-	PCR (copies/µg DNA)
								brain	-	
								gonads	-	
								heart	-	
								kidney	-	
								liver	-	
								lung	-	
								axillary lymph node	$2x10^4$ (4/6)	
								cranial MLN	-	
								posterior MLN	-	
								spleen	-	
								wound bed	$1.4x10^8$ (6/6)	
								wound bed	$2.7x10^4$	RT-PCR (PDGF-B)
								axillary lymph node	$5x10^2$	
Gu et al., 2004	Ad5	rabbit (New Zealand White)	?	dermal (wound application)	$7x10^{11}$ vp, (4 weekly doses)		22 days	blood	-	PCR (copies/µg DNA)
								brain	-	
								gonads	-	
								heart	-	
								kidney	-	
								liver	-	
								lung	-	
								axillary lymph node	$4.5x10^4$ (6/6)	
								cranial MLN	-	
								posterior MLN	-	
								spleen	-	
								wound bed	$9.4x10^8$ (6/6)	
								wound bed	$1.8x10^4$	RT-PCR (PDGF-B)
								axillary lymph node	$7.6x10^2$	

Table B.5. Intra-prostatic administration of Ad

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	results	analysis technique	time point	tested compartments	results	analysis technique		
Johnson et al., 2006	Ad5	mice (SCID)	male	intra-prostatic	$1x10^8$ pfu	14 days	14 days	lung liver kidney spleen prostate	2.7% 100% 1% 5.5% 1189%	RT-PCR						
								lung liver kidney spleen prostate	1.4% 100% 0.7% 0.3% 156%	in vivo optical imaging						
									expressed relative to liver							
Trudel et al., 2003	Ad5 (AdCAIL-2)	humans (patients with prostate cancer)	male (n = 12)	intra-prostatic	$1x10^9$ – $1x10^{10}$ pfu	3 months	1 month	blood	-	PCR	?	saliva stool	- -	PCR		
							1 month	prostate	+ (1/4)							
Freytag et al., 2003	Ad5	human (patients with adenocarcinoma of the prostate)	male (n = 15)	intra-prostatic	$1x10^{12}$ vp	1 year	45 days > 45 days	blood	+ -	PCR						
Kubo et al., 2003	Ad5	human (patients with prostate cancer)	male (n = 11)	intra-prostatic (prostate bed)	$2.5x10^8$ pfu	10 days	2 days	serum	0%	cell culture	2 days 3 days	urine	100% 0%	cell culture		
Kubo et al., 2003	Ad5	human (patients with prostate cancer)	male (n = 11)	intra-prostatic (prostate bed)	$2.5x10^9$ pfu	10 days	2 days	serum	0%	cell culture	2 days 3-10 days	urine	100% 0%	cell culture		
Barton et al., 2003	Ad5	dogs	male	intra-prostatic	$3x10^8$ – $1x10^{12}$ vp		1-5 days	thyroid salivary glands stomach duodenum bladder prostate	+ + + + + +	Isotope imaging						in control dogs, isotope uptake was present in thyroid, stomach and urinary bladder, but not prostate
							7 days	thyroid salivary glands stomach duodenum bladder prostate	+ + + + + -							
							2 days	muscle heart prostate rectum epididymus/testes spleen kidney	+ + + + + + +	Isotope quantification (10^4 – 10^7 cps/g tissue)						

							liver lung bladder stomach thyroid	+ + + + +					
Herman et al., 1999	Ad5	human (patients with recurrent prostate cancer)	male	intra-prostatic	$1x10^{8}$ iu (n=4)	1-4 days	serum	-	culture for wt Ad	1-4 days 1-4 days	ear swab nasal swab urine	- - -	culture for wt Ad PCR
Herman et al., 1999	Ad5	human (patients with recurrent prostate cancer)	male	intra-prostatic	$1x10^{9}$ iu (n=5)	1-4 days	serum	-	culture for wt Ad	1-4 days 1 day 2-5 days	ear swab nasal swab urine	- - + -	culture for wt Ad PCR
Herman et al., 1999	Ad5	human (patients with recurrent prostate cancer)	male	intra-prostatic	$1x10^{10}$ iu (n=4)	1-4 days	serum	-	culture for wt Ad	1-4 days 1-11 days 12-15 days	ear swab nasal swab urine	- - + -	culture for wt Ad PCR
Herman et al., 1999	Ad5	human (patients with recurrent prostate cancer)	male	intra-prostatic	$1x10^{11}$ iu (n=5)	1-4 days	serum	-	culture for wt Ad	1-4 days 1-32 days 33-36 days	ear swab nasal swab urine	- - + -	culture for wt Ad PCR

Table B.6. Administration into the brain of Ad

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	results	analysis technique	time point	tested compartments	results	analysis technique		
Immonen et al., 2004	Ad5	human (patients with malignant glioma)	male + female (n = 17)	healthy brain tissue of wound bed after tumor resection	$3x10^{10}$ pfu		3 days	plasma	+ (2/17)	PCR						
Germano et al., 2003	Ad5	human (patients with glioblastoma)	male and female (n = 11)	intra-tumoral (brain)	$2.5x10^{11}$ $3 x10^{11}$ $9 x10^{11}$ $2.7 x10^{12}$ $5.6x10^{12}$ vp	248 weeks	24 h	blood	- -	IF techniques PCR	24 h	nasal swab urine nasal swab urine	- - - -	IF techniques PCR		
Lang et al., 2003	Ad5	human (patients with malignant glioma)	male and female (n = 15)	brain (intra-cranial)	$3x10^{10}$ $3 x10^{11}$ $1 x10^{12}$ $3x10^{12}$ vp	21 months	24 h, 2 weeks, 1 month	plasma	-	cell culture	24 h, 2 weeks, 1 month	urine sputum rectal samples	- - -	cell culture		
Smitt et al., 2003	Ad5	human (patients with malignant glioma)	male and female (n = 14)	brain (intra-cranial)	$4.6x10^{8}$, $4.6x10^{9}$, $4.6x10^{10}$, $4.6x 10^{11}$ vp	29 months	1-2 days	blood	-	cell culture	1-2 days	nasal swab urine faeces	- - -	cell culture		
Sandmair et al., 2000	Ad5	human (patients with malignant glioma)	male and female (n = 12)	healthy brain tissue of wound bed after tumor resection	$3x10^{8}$ - $3x10^{10}$ pfu		3, 5, 7, 21 days	plasma	0%	PCR	3, 5, 7, 21 days	urine	0%	PCR		

Table B.7. Intra-muscular administration of Ad

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	results	analysis technique	time point	tested compartments	results	analysis technique		
Matyas et al., 2005	Ad5	human (CLI patients)	male and female (n = 10)	intra-muscular	$2.87x10^8$, $2.87x10^9$, $2.87x10^{10}$ vp	12 weeks	2 days	blood	-	infectivity assay	2 days	faeces	-	infectivity assay		
							7 days		-		7 days		-			
							2 weeks		-		2 weeks		-			
							4 weeks		-		4 weeks		-			
							8 weeks		-		8 weeks		-			
							12 weeks		-		12 weeks		-			
											2 days	urine	-			
											7 days		-			
											2 weeks		-			
											4 weeks		-			
											8 weeks		-			
											12 weeks		-			
											2 days	throat swabs	-			
											7 days		-			
											2 weeks		-			
											4 weeks		-			
											8 weeks		-			
											12 weeks		-			
Grines et al., 2002	Ad5	human (patients with chronic satble agina)	male and female (n=60)	intra-coronary	$3.3x10^8$ $1x10^9$ $3.3x10^9$ $1x10^{10}$ vp	12 weeks	0 h (during infusion)	pulmonary artery blood	250 - $2.3x10^4$ IFU/mL and ~22 - ~90% of patients (lowest dose to highest dose, respectively)	infectious assay	?	urine	-	PCR		
					$6.4x10^6$ $2.0x10^8$ $1.9x10^8$ $8.8x10^8$ iu		1 h	venous blood	~12~% of patients positive from dose $3.3x10^9$ vp up to 38% of patients from dose $3.3x10^{10}$ vp		8 weeks	semen	-			
Hackett et al., 2000	Ad5	pig (Yorkshire)	male	intra-myocardial (n=3)	10^{12} vp (divided over 10 injections)	24 h	1 h	left ventricle	mean 6.2 (0.019-50)	Q-PCR (vc/cellular genome)						
							24 h		mean 0.44							
							24 h	lung	36	Q-PCR (% of total recovered)						
								liver	6							
								spleen	7							
								left ventricle	50							

Hackett et al., 2000	Ad5	pig (Yorkshire)	male	intra-coronary (n=3)	10^{12} vp	24 h	1 h	left ventricle	mean 0.24 (0.0012-2.29)	Q-PCR (vc/cellular genome)					
							24 h	left ventricle	mean 0.044						
							24 h	lung	74	Q-PCR (% of total recovered)					
								liver	15						
								spleen	3						
								left ventricle	8						
Varenne et al., 2000	Ad5 (with wt E3 region)	pig		intra-coronary	$1.5x10^{9}$ pfu	28 days					2 and 7 days	urine stool	- -	cell culture	

Table B.8. Inhalatory and intranasal-bronchial administration of Ad

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	results	analysis technique	time point	tested compartments	results	analysis technique		
Perricone et al., 2001	Ad2	human (patients with cystic fibrosis)	? (n = 6)	intra-lobar	$8x10^7$ iu		7 days	cytology brush sample	-	RT-PCR						suspected degradation in some samples
Perricone et al., 2001	Ad2	human (patients with cystic fibrosis)	? (n = 6)	intra-lobar	$2.5x10^8$ iu		7 days		-	RT-PCR/PCR						suspected degradation in some samples
Perricone et al., 2001	Ad2	human (patients with cystic fibrosis)	? (n = 6)	intra-lobar	$8x10^8$ iu		2 days 7 days	cytology brush sample	2/2 1/1	PCR						suspected degradation in some samples
							2 days 7 days	cytology brush sample	0/2 0/1	RT-PCR						
Perricone et al., 2001	Ad2	human (patients with cystic fibrosis)	? (n = 6)	intra-lobar	$2.5x10^9$ iu		2 days	cytology brush sample	2/3 3/3 5.6% + cells	PCR RT-PCR FISH						suspected degradation in some samples
Perricone et al., 2001	Ad2	human (patients with cystic fibrosis)	? (n = 2)	inhalation (aerosol)	$8x10^6$ iu		7 days	cytology brush sample	-	PCR						suspected degradation in some samples
Perricone et al., 2001	Ad2	human (patients with cystic fibrosis)	? (n = 2)	inhalation (aerosol)	$2.5x10^7$ iu		2 days 7 days	cytology brush sample	1/1 0/1	PCR						suspected degradation in some samples
							2 days 7 days		0/2 0/1	RT-PCR						
Perricone et al., 2001	Ad2	human (patients with cystic fibrosis)	? (n = 2)	inhalation (aerosol)	$8x10^7$ iu		2 days	cytology brush sample	2/2 2/2 3.1% + cells	PCR RT-PCR FISH						suspected degradation in some samples
Perricone et al., 2001	Ad2	human (patients with cystic fibrosis)	? (n = 2)	inhalation (aerosol)	$2.5x10^8$ iu		2 days	cytology brush sample	2/2 2/2 4% + cells	PCR RT-PCR FISH						suspected degradation in some samples
Perricone et al., 2001	Ad2	human (patients with cystic fibrosis)	? (n = 2)	inhalation (aerosol)	$8x10^8$ iu		2 days	cytology brush sample	2/2 0/2 2.7% + cells	PCR RT-PCR FISH						suspected degradation in some samples
Perricone et al., 2001	Ad2	human (patients with cystic fibrosis)	? (n = 2)	inhalation (aerosol)	$2.5x10^9$ iu		2 days	cytology brush sample	2/2 0/2 3.7% + cells	PCR RT-PCR FISH						suspected degradation in some samples
							7 days		1/1 0/1	PCR RT-PCR						
Perricone et al., 2001	Ad2	human (patients with cystic fibrosis)	? (n = 2)	inhalation (aerosol)	$8x10^9$ iu		2 days	cytology brush sample	2/2 0/2 5.3% + cells	PCR RT-PCR FISH						suspected degradation in some samples
							7 days		1/1 0/1	PCR RT-PCR						
Perricone et al., 2001	Ad2	human (patients with cystic fibrosis)	? (n = 2)	inhalation (aerosol)	$2.5x10^{10}$ iu		2 days	cytology brush sample	2/2 0/2 2% + cells	PCR RT-PCR FISH						suspected degradation in some samples
							7 days		1/2 0/2	PCR RT-PCR						

Zabner et al., 1996	Ad2	human (cystic fibrosis patients)	male and female (n=6)	intra-nasal	successive doses of ($2x10^7$),$2x10^8$, $2x10^9$, $6x10^9$ and 10^{10} IU						24 h	nasal swab sputum rectal swab urine	+ (1/6) - - -	culture
											>24 h	nasal swab sputum rectal swab urine	- - - -	
Crystal et al., 1994	Ad2	human (cystic fibrosis patients)	male and female (n=4)	intra-nasal and intra-bronchial			?	blood	-	culture	?	urine pharyngeal rectal swabs nasal swabs	- - - -	culture
Zuckerman et al., 1999	Ad5	human (cystic fibrosis patients)	male and female (n=11)	broncho instillation	$2.1x10^9$ - $2.1x10^{11}$ vp		4 days	bronchal brushes	+	in situ hybridisation	1 + 2 days	urine sputum rectal swabs nasopharynx swabs	- - - -	Ad focus-forming assay
Worgall et al., 1997	Ad5	mice (C57BL/6)	male and female	intra-tracheal	10^9 pfu	24 h	10 min 2 h 8 h 24 h	lung	5.5 4 3.5 2.5	Southern blot				
Worgall et al., 1997	Ad5	mice (BALB/c)	male and female	intra-tracheal	10^9 pfu	24 h	10 min 24 h		1.5 0.5	Southern blot				
Worgall et al., 1997	Ad5	mice (athymic immuno-deficient BALB/c nu/nu)	male and female	intra-tracheal	10^9 pfu	24 h	10 min 24 h		1.5 0.4	Southern blot				
Bellon et al., 1997	Ad5	human (cystic fibrosis patients)	male and female (n=6)	nasal instillation + lung aerosolation	$10^5 + 10^7$ pfu		? 1 day 3 days 7 days 14 days 21 days 28 days	blood blood	- - - - - - -	PCR infectious assay	3 days 7/8 days 14/15days 21/22days 3 days 7/8 days 14/15days 21/22days 1 day 3 days 7/8 days 14/15days 21/22days 1 day 2 days 3 days 4 days 7/8 days 9 days 14/15days 21/22days	BAL bronchial brush nasal brush saliva 	+ (2/2) - (0/2) - (0/1) - (0/1) - (0/2) + (1/2) - (0/1) - (0/1) + (2/2) - (0/2) - (0/2) - (0/1) + (1/2) + (1/2) - (0/2) - (0/2) - (0/2) + (1/2) + (1/2) - (0/2) - (0/1)	PCR

										1 day	tonsils	- (0/1)	
										2 days		- (0/2)	
										3 days		- (0/2)	
										4 days		- (0/2)	
										7/8 days		- (0/2)	
										9 days		- (0/2)	
										14/15days		- (0/2)	
										21/22days		- (0/2)	
										1 day	tonsils, nasal	-	infectious
										3 days	and bronchial	-	assay
										7 days	brush, BAL,	-	
										14 days	stools, urine and	-	
										21 days	saliva	-	
										28 days		-	
Bellon et al., 1997	Ad5	human (cystic fibrosis patients)	male and female (n=6)	nasal instillation + lung aerosolation	$10^7 + 10^8$ pfu	48 h	blood	+	PCR	3 days	BAL	+ (1/1)	PCR
						> 48 h		-		4 days		+ (1/1)	
										7/8 days		+ (1/2)	
						1 day	blood	-	infectious	14/15days		- (0/2)	
						3 days		-	assay				
						7 days		-		3 days	bronchial brush	- (0/1)	
						14 days		-		4 days		+ (1/1)	
						21 days		-		7/8 days		+ (1/2)	
						28 days		-		14/15days		- (0/2)	
										1 day	nasal brush	+ (2/2)	
										3 days		+ (1/1)	
										4 days		+ (1/1)	
										7/8 days		- (0/2)	
										14/15days		+ (1/2)	
										21/22days		- (0/2)	
										1 day	saliva	+ (2/2)	
										2 days		+ (1/2)	
										3 days		- (0/2)	
										4 days		+ (1/2)	
										7/8 days		+ (1/2)	
										14/15days		- (0/2)	
										21/22days		- (0/2)	
										1 day	tonsils	- (0/2)	
										2 days		- (0/2)	
										3 days		- (0/2)	
										4 days		+ (1/2)	
										7/8 days		- (0/2)	
										14/15days		- (0/2)	
										21/22days		- (0/2)	
										1 day	tonsils, nasal	-	infectious
										3 days	and bronchial	-	assay
										7 days	brush, BAL,	-	
										14 days	stools, urine and	-	
										21 days	saliva	-	
										28 days		-	

Bellon et al., 1997	Ad5	human (cystic fibrosis patients)	male and female (n=6)	nasal instillation + lung aerosolation	$4x10^8$ + $5.4x10^8$ pfu	?	blood	-	PCR	3 days	BAL	+ (2/2)	PCR
										7/8 days		+ (1/2)	
						1 day	blood	-	infectious	14/15days		- (0/2)	
						3 days		-	assay				
						7 days		-		3 days	bronchial brush	- (0/2)	
						14 days		-		7/8 days		- (0/2)	
						21 days		-		14/15days		+ (1/2)	
						28 days		-					
										1 day	nasal brush	+ (2/2)	
										3 days		+ (1/2)	
										7/8 days		+ (1/2)	
										14/15days		+ (1/2)	
										21/22days		- (0/2)	
										1 day	saliva	+ (1/2)	
										2 days		- (0/2)	
										3 days		- (0/2)	
										4 days		- (0/2)	
										7/8 days		- (0/2)	
										9 days		- (0/2)	
										14/15days		- (0/2)	
										21/22days		- (0/2)	
										1 day	tonsils	+ (1/2)	
										2 days		- (0/2)	
										3 days		- (0/2)	
										4 days		- (0/2)	
										7/8 days		- (0/2)	
										9 days		- (0/2)	
										14/15days		- (0/2)	
										21/22days		- (0/2)	
										1 day	tonsils, nasal	-	infectious
										3 days	and bronchial	-	assay
										7 days	brush, BAL,	-	
										14 days	stools, urine and	-	
										21 days	saliva	-	
										28 days		-	
Knowles et al., 1995	Ad5	human (cystic fibrosis patients)	male and female (n=12)	intra-nasal	$2x10^7$ pfu					2 – 8 days	dosed nostril	-	PCR
										1 – 4 days	control nostril	-	
										1 – 4 days	pharynx swab	-	
										1 – 4 days	urine	+ (1/3) for wt Ad	
										20 min	dosed, nostril	+	infectious assay
										1 – 4 days	rectal swab	-	

Knowles et al., 1995	Ad5	human (cystic fibrosis patients)	male and female (n=12)	intra-nasal	$2x10^8$ pfu	2 – 8 days	dosed nostril	-	PCR	
						1 – 4 days	control nostril	+ (1/3)		
						1 – 4 days	pharynx swab	-		
						1 day	urine	+ (1/3) for ? Ad		
						2 – 4 days		-		
						20 min	dosed nostril	+	infectious assay	
						1 – 4 days	rectal swab	-		
Knowles et al., 1995	Ad5	human (cystic fibrosis patients)	male and female (n=12)	intra-nasal	$2x10^9$ pfu	2 – 8 days	dosed nostril	+	PCR	
						1 – 4 days	control nostril	-		
						1 + 2 days	pharynx swab	+ (1/3)		
						3 + 4 days		-		
						1 – 4 days	urine	+ (1/3) for wt Ad		
						20 min	dosed nostril	+	infectious assay	
						1 – 4 days	rectal swab	-		
Knowles et al., 1995	Ad5	human (cystic fibrosis patients)	male and female (n=12)	intra-nasal	$2x10^{10}$ pfu	20 min	dosed nostril	+	infectious assay	
						1 day	control nostril	+ (1/3)		
						1 day	rectal swab	+		
						2 days		+		
						2 – 8 days	dosed nostril	+	PCR	
						1 – 4 days	control nostril	+ (1/3)		
						1 + 2 days	pharynx swab	+ (1/3)		
						3 + 4 days		-		
						1 – 4 days	urine	-		

Table B.9. Ocular administration of Ad

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	results	analysis technique	time point	tested compartments	results	analysis technique		
Veneziale et al., 2007	Ad5 (p21) (SCH 412499)	monkey (Cynomolgus)	male (n = 4) and female (n = 4)	ocular (sub-conjuctival)	$7.5x10^{10}$ vp	29 days	15 min 1 h 2 h 20-29 h 96 h	whole blood (surgery group)	91000±160000 17000±24000 19000±31000 26000±26000 270±680	PCR (copies/ml blood)						
							2 h 24 h 4 days 7 days 29 days	whole blood (non-surgery group)	6300±16000 37000±49000 2200±3000 490±910 0	PCR (copies/ml blood)						
							6 days	bone marrow liver spleen heart kidney lung ovary/testis left conjunctiva/sclera right conjunctiva/sclera left optic nerve right optic nerve left retina right retina left LGN right LGN occipital optic chiasma	2/6 (18 gc) 4/6 (490 gc) 4/6 (1500 gc) 2/6 (87 gc) 3/6 (16 gc) 0/6 0/6 6/6 ($3.9x10^6$ gc) 6/6 ($1.7x10^6$ gc) 5/6 (1100 gc) 5/6 (51510 gc) 2/6 (420 gc) 0/6 0/6 1/6 (25) 0/6 0/6	PCR					
							29 days	bone marrow liver spleen ovary/testis	0/6 3/6 (74 gc) 3/6 (280 gc) 0/6							
Campochiaro et al., 2006	Ad5	human (AMD patients)	male and female (n = 88)	ocular (intra-vitreous)	$1x10^6$, $1x10^{6.5}$, $1x10^7$, $1x10^{7.5}$, $1x10^8$, $1x10^{8.5}$, $1x10^9$, $1x10^{9.5}$ pfu	12 months					3 weeks	sputum urine	- -	culture		

Table B.10. Salivary gland administration of Ad

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	results	analysis technique	time point	tested compartments	results	analysis technique		
Zheng et al., 2006a	Ad5	rats (F344)	male (n = 5) and female (n = 5)	retroductal delivery to right submandibular gland	$2x10^{11}$ vp	92 days	3 days	brain	-	RT-PCR	3 days	saliva	1/10			wild type Ad was not found in blood (0/43) or saliva (0/50) (RT-PCR)
							15 days		-		15 days		-			
							29 days		-		29 days		-			
							57 days		-		57 days		-			
							92 days		-		92 days		-			
							3 days	left parotid gland	-							
							15 days		-							
							29 days		-							
							57 days		-							
							92 days		-							
							3 days	left submandibular gland	4/10							
							29 days		1/10							
							57 days		2/10							
							92 days		3/10							
							3 days	buccal oral mucosa	-							
							15 days		-							
							29 days		-							
							57 days		-							
							92 days		-							
							3 days	left sublingual gland	-							
							15 days		2/10							
							29 days		-							
							57 days		-							
							92 days		1/10							
							3 days	right submandibular gland	9/10							
							15 days		5/10							
							29 days		4/10							
							57 days		5/10							
							92 days		4/10							
							3 days	floor-of-mouth oral mucosa	3/10							
							15 days		-							
							29 days		-							
							57 days		-							
							92 days		-							
							3 days	right sublingual gland	2/10							
							15 days		5/10							
							29 days		3/10							
							57 days		3/10							
							92 days		7/10							
							3 days	spleen	2/10							
							15 days		2/10							
							29 days		-							
							57 days		-							

92 days		1/10
3 days	heart	-
15 days		-
29 days		-
57 days		-
92 days		-
3 days	lung	2/10
15 days		-
29 days		-
57 days		-
92 days		-
3 days	small intestine	-
15 days		-
29 days		-
57 days		-
92 days		-
3 days	kidney	-
15 days		-
29 days		-
57 days		-
92 days		-
3 days	gonads	-
15 days		1/10
29 days		-
57 days		-
92 days		-
3 days	large intestine	-
15 days		-
29 days		-
57 days		-
92 days		-
3 days	palatal oral mucosa	-
15 days		-
29 days		-
57 days		-
92 days		-
3 days	tongue	-
15 days		-
29 days		-
57 days		-
92 days		-
3 days	liver	1/10
15 days		1/10
29 days		-
57 days		-
92 days		-
3 days	left mandibular	1/10

						15 days	lymph node	1/10			
						29 days		-			
						57 days		-			
						92 days		1/10			
						3 days	right mandibular	2/10			
						15 days	lymph node	-			
						29 days		-			
						57 days		-			
						92 days		1/10			
						3 days	right parotid gland	1/10			
						15 days		-			
						29 days		-			
						57 days		-			
						92 days		-			
						3 days	blood	-			
						15 days		-			
						29 days		-			
						57 days		-			
						92 days		-			
Zheng et al., 2006b	Ad5	rat (F344)	male (n = 12) and female (n = 12)	retroductal delivery to right submandibular gland	$2.4x10^8$ vp	3 days	heart	0/6	Q-PCR		replication competent Ad was not found in blood and saliva on 3 days (Q-PCR, all groups)
							lung	0/8			
							liver	0/8			
							spleen	0/6			
							gonads	0/8			
							blood	0/7			
							right submandibular gland	8/8			
							left submandibular gland	1/4			
						29 days	heart	0/15			
							lung	0/12			
							liver	0/11			
							spleen	1/13			
							gonads	0/14			
							blood	0/13			
							right submandibular gland	6/9			
							left submandibular gland	0/11			
Zheng et al., 2006b	Ad5	rat (F344)	male (n = 12) and female (n = 12)	retroductal delivery to right submandibular gland	$6x10^9$ vp	3 days	heart	0/8	Q-PCR		replication competent Ad was not found in blood and saliva on 3 days (Q-PCR, all groups)
							lung	1/7			
							liver	1/8			
							spleen	1/7			
							gonads	0/8			
							blood	0/7			
							right submandibular gland	6/6			
							left submandibular gland	4/6			
						29 days	heart	3/15			
							lung	2/13			
							liver	3/15			

							spleen	4/12		
							gonads	0/12		
							blood	2/15		
							right submandibular gland	9/12		
							left submandibular gland	5/8		
Zheng et al., 2006b	Ad5	rat (F344)	male (n = 12) and female (n = 12)	retroductal delivery to right submandibular gland	$1.5x10^{11}$ vp	3 days	heart	0/5	Q-PCR	replication competent Ad was not found in blood and saliva on 3 days (Q-PCR, all groups)
							lung	1/6		
							liver	1/4		
							spleen	3/4		
							gonads	0/7		
							blood	1/5		
							right submandibular gland	8/8		
							left submandibular gland	8/8		
						29 days	heart	0/14		
							lung	1/9		
							liver	2/13		
							spleen	3/6		
							gonads	1/13		
							blood	0/13		
							right submandibular gland	6/8		
							left submandibular gland	10/11		

Table B.11. Other routes of administration of Ad

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	results	analysis technique	time point	tested compartments	results	analysis technique		
Kubo et al., 2003	Ad5	human (patients with prostate cancer)	male (n = 11)	para-aortic lymph node	$2.5x10^8$ pfu	10 days	2 days	serum	0%	cell culture	2 days	urine	0%	cell culture		
Kubo et al., 2003	Ad5	human (patients with prostate cancer)	male (n = 11)	para-aortic lymph node	$2.5 x10^{10}$ pfu	10 days	2 days 3 days 7-10 days	serum	100% 50% 0%	cell culture	2 days 3 days 7-10 days	urine	0% 0% 0%	cell culture		
Kubo et al., 2003	Ad5	human (patients with prostate cancer)	male (n = 11)	spine	$2.5x10^8$ pfu	10 days	2 days	serum	0%	cell culture	2 days	urine	0%	cell culture		
Kubo et al., 2003	Ad5	human (patients with prostate cancer)	male (n = 11)	spine	$2.5x10^{10}$ pfu	10 days	2 days 3 days 7-10 days	serum	100% 50% 0%	cell culture	2-10 days	urine	0%	cell culture		
Kubo et al., 2003	Ad5	human (patients with prostate cancer)	male (n = 11)	ileum	$2.5x10^9$ pfu	10 days	2 days	serum	0%	cell culture	2-10 days	urine	0%	cell culture		
Kubo et al., 2003	Ad5	human (patients with prostate cancer)	male (n = 11)	ileum	$2.5x10^{10}$ pfu	10 days	2 days 3-10days	serum	100% 0%	cell culture	2-10 days	urine	0%	cell culture		
Senoo et al., 2000	Ad5	mice (ICR)	male and female	foetal in utero (amniotic fluid)	$3x10^6$ pfu	72 h	72 h	lung skin surface digestive epithelium	+ + +	β-gal staining						
Senoo et al., 2000	Ad5	mice (ICR)	male and female	foetal intra-peritoneal space foetus	$3x10^6$ pfu	72 h	72 h	peritoneum upper digest tract	+ +							
Senoo et al., 2000	Ad5	mice (ICR)	male and female	foetal placental parenchyma	$3x10^6$ pfu	72 h	72 h	foetal organs	-							
Senoo et al., 2000	Ad5	guinea pig (Hartley)	male and female	foetal intra-venous (umbilical vein)	$3x10^8$ pfu	24 h	24 h	liver heart spleen adrenal gland kidney small intestine brainstem cerebral cortex lung muscle placenta	>80% cells + ~30% cells + ~30% cells + + + + - - - - +	β-gal staining						
								liver heart spleen adrenal gland kidney small intestine brainstem cerebral cortex lung muscle	+++++ ++ +++ ++ + + + - + +	Q-PCR (copies/µg DNA)						

								placenta	+	
Wood et al., 1999	Ad5	mice (BALB/c)	female	intra-vesical (bladder)	$5x10^9$ pfu	21 days	1 day 3 days 7 days 14 days 21 days	bladder	50-$2x10^4$ 300-5000 0-1000 10-2000 20-500	luciferase assay (RLU/mg)
							1 day 3 days 7 days 14 days 21 days	bladder	≥2500 0-250 0-250 0-250 0-250	PCR (vector copies)
							24 h	bladder liver kidney heart ovary ureter adrenal lung	50-$2x10^4$ - 0-20 0-3 - - 0-3 -	luciferase assay (RLU/mg)
							24 h	bladder liver kidney heart ovary ureter adrenal lung	≥2500 0-25 0 0-25 0-25 0-250 0 0-25	PCR (vector copies)

Table B.12. Intravenous and intra-arterial administration of AAV

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	results	analysis technique	time point	tested compartments	results	analysis technique		
Grimm et al., 2003	AAV1	mice (C57BL/6)	? (n = 3)	intra-venous (portal vein and tail vein)	$2x10^{11}$ gc		6 weeks	liver	+	Q-PCR and Southern blot						ratio portal/tail vein measured with Q-PCR: is 8.2
								heart	+							
								kidney	+							
								spleen	+							
								brain	+							
								lung	+							
Jacobson et al., 2006b	AAV2 (RPE65)	monkey (Cynomolgus)	male (n = 2)	intra-venous	$1.5x10^{12}$ – $4.5x10^{12}$ vg		3 months	heart	-	antibody assay						
								lung	+ (1/2)							
								liver	-							
								pancreas	-							
								spleen	-							
								kidney	-							
								jejunum	-							
								gonads	-							
								skeletal muscle	-							
								preauricular lymph node	-							
								mandibular lymph node	-							
								tracheobronchial lymph node	-							
								mesenteric lymph node	-							
								left optic nerve	-							
								left orbital tissue	-							
								right optic nerve	-							
								right orbital tissue	-							
								optic chiasm	-							
								left optic tract	-							
								left LGN	-							
								left optic radiation	-							
								left visual cortex	-							
								left superior colliculus	-							
								right optic tract	-							
								right LGN	-							
								right optic radiation	-							
								right visual cortex	-							
								right superior colliculus	-							
								left thalamus	-							
								right thalamus	-							
								cerebellum	-							
Mori et al., 2006	AAV2 (β-galF)	monkey (Cynomolgus)	female (n = 1)	intra-venous	$2.5x10^{10}$ gc/kg	3 months	2 days	cerebrum	+	PCR						
								cerebellum	+							
								bone marrow	++							
								retina	-							
								skin	-							
								muscle	-							
								trachea	+							
								lung	++							
								heart	-							
								liver	++							
								gallbladder	++							
								pancreas	+							
								spleen	++++							
								oesophagus	+							
								stomach	+							
								ileum	+							
								colon	+							

								kidney	-	
								adrenal gland	-	
								bladder	+	
								tonsil	+++	
								thymus	+	
								parotid gland	+	
								submandibular gland	+	
								thyroid gland	++	
								axillary lymph node	+++	
								hilar lymph node	+	
								mesenteric lymph node	++	
								iliac lymph node	++	
								inguinal lymph node	+	
								testis/ovary	+	
								epididymis/uterus	+	
							3 months	cerebrum	-/+	
								cerebellum	+	
								bone marrow	-/+	
								retina	-	
								skin	+/-	
								muscle	-	
								trachea	+/-	
								lung	-/+	
								heart	-/+	
								liver	-/+	
								gallbladder	+/-	
								pancreas	+/-	
								spleen	++	
								oesophagus	-	
								stomach	-/+	
								jejunum	+/-	
								ileum	+/-	
								colon	+	
								kidney	+/-	
								adrenal gland	-	
								bladder	-/+	
								tonsil	+/-	
								thymus	-	
								parotid gland	-	
								submandibular gland	-	
								thyroid gland	+/-	
								axillary lymph node	-/+	
								hilar lymph node	-/+	
								mesenteric lymph node	-/+	
								inguinal lymph node	+/-	
								testis/ovary	+/-	
								epididymis/uterus	-/+	
Mori et al., 2006	AAV2 (EGFPtub)	monkey (Cynomolgus)	male (n = 2) and female (n = 2)	intra-venous	$2.5x10^{11}$ gc/kg	3 months	3 months	cerebrum	-/+	PCR
								cerebellum	-	
								spinal cord	-	
								bone marrow	-/++	
								skin	-	
								muscle	-/++	
								trachea	+	
								lung	-/+	
								heart	+/-	
								liver	++	
								gallbladder	+	
								pancreas	-/+	
								spleen	+++	
								oesophagus	-/+	
								stomach	-	

								jejunum	-	
								ileum	-	
								colon	-/+	
								kidney	+/-	
								adrenal gland	-/++	
								bladder	-/+	
								tonsil	++	
								thymus	-	
								parotid gland	-/++	
								submandibular gland	-	
								thyroid gland	-	
								axillary lymph node	++	
								hilar lymph node	-/++++	
								mesenteric lymph node	+	
								iliac lymph node	-/+++	
								inguinal lymph node	++	
								testis/ovary	-	
								epididymis/uterus	-	
Mori et al., 2006	AAV2 (EGFPtub)	monkey (Cynomolgus)	male (n = 2) and female (n = 2)	intra-venous	$1.0x10^{11}$ gc/kg	5 months	5 months	cerebrum	-	PCR
								cerebellum	-	
								bone marrow	-/+	
								skin	-	
								muscle	-/+	
								trachea	-	
								lung	+/-	
								heart	+/-	
								liver	++	
								gallbladder	-/++	
								pancreas	-/++	
								spleen	+++	
								oesophagus	-	
								stomach	-	
								jejunum	-	
								ileum	-/+	
								colon	-/+	
								kidney	+/-	
								adrenal gland	-/+	
								bladder	-/+	
								tonsil	+	
								thymus	-/+	
								parotid gland	-	
								submandibular gland	-/+	
								thyroid gland	-/+++	
								axillary lymph node	-/++++	
								mesenteric lymph node	-/+	
								iliac lymph node	-/+++	
								inguinal lymph node	+	
								testis/ovary	-	
								epididymis/uterus	-/+	
Mori et al., 2006	AAV2	monkey (Cynomolgus)	male (n = 1) and female (n = 2	intra-venous	$1.0x10^{10}$ gc/kg	7 months	3 months	cerebrum	-	PCR
								cerebellum	-	
								bone marrow	-	
								skin	-	
								retina	-	
								muscle	-	
								trachea	-	
								lung	-	
								heart	-	
								liver	+ (1/2)	
								gallbladder	-	
								pancreas	-	
								spleen	++ (2/2)	
								oesophagus	-	

	stomach	-
	jejunum	-
	ileum	+ (1/2)
	colon	+ (2/2)
	kidney	-
	adrenal gland	-
	bladder	++ (1/2)
	tonsil	+ (2/2)
	thymus	-
	parotid gland	-
	submandibular gland	+ (1/2)
	thyroid gland	-
	axillary lymph node	+ (2/2)
	hilar lymph node	+ (1/2)
	mesenteric lymph node	+ (1/2)
	iliac lymph node	+ (1/2)
	inguinal lymph node	+ (2/2)
	testis/ovary	-
	epididymis/uterus	-
7 months (n=1)	cerebrum	-
	cerebellum	-
	bone marrow	-
	skin	-
	retina	+
	muscle	-
	trachea	-
	lung	-
	heart	-
	liver	-
	gallbladder	-
	pancreas	-
	spleen	++
	oesophagus	-
	stomach	-
	jejunum	-
	ileum	-
	colon	+
	kidney	-
	adrenal gland	-
	bladder	-
	tonsil	-
	thymus	-
	parotid gland	-
	submandibular gland	-
	thyroid gland	-
	axillary lymph node	+
	hilar lymph node	+
	mesenteric lymph node	+
	iliac lymph node	+
	inguinal lymph node	-
	testis/ovary	-
	epididymis/uterus	-

Schuet-trumpf et al., 2006	AAV2	rabbit	male	intra-venous	$1x10^{11}$ vg/kg	18-20 months	gonads liver	- 744±546 vector copies/µg DNA	PCR	4 days 1 week 2 weeks 3 weeks 4 weeks 6 weeks 8 weeks 13 weeks 18 weeks 23 weeks 28 weeks 12 months 18 months	semen	+ + - + - + - - - - - - -	infectious assay PCR	the vector clearance from semen can be accelerated by increasing the frequency of semen collection/
Schuet-trumpf et al., 2006	AAV2	rabbit	male	intra-venous	$1x10^{12}$ vg/kg	18-20 months	gonads liver	+ 6654±4018 vector copies/µg DNA	PCR	4 days 1 week 2 weeks 3 weeks 4 weeks 6 weeks 8 weeks 13 weeks 18 weeks 23 weeks 28 weeks 12 months 18 months	semen	+ + + + + + + - - - - - -	infectious assay PCR	the vector clearance from semen can be accelerated by increasing the frequency of semen collection/
Schuet-trumpf et al., 2006	AAV2	rabbit	male	intra-venous	$1x10^{13}$ vg/kg	18-20 months	gonads liver	+ 130213±57976 vector copies/µg DNA	PCR	4 days 1 week 2 weeks 3 weeks 4 weeks 6 weeks 8 weeks 13 weeks 18 weeks 23 weeks 28 weeks 12 months 18 months	semen	+ + + + + + + + - - - - -	infectious assay PCR	the vector clearance from semen can be accelerated by increasing the frequency of semen collection/
Ohashi et al., 2005	AAV2	rat (nude and Lewis)	male	hepatic artery infusion	$5x10^{12}$ vg/kg	7 weeks	liver	0.02-0.05 vc	Southern blot					Values determined from figure
Ohashi et al., 2005	AAV2	rat (nude and Lewis)	male	hepatic artery infusion without outflow block	$5x10^{12}$ vg/kg	7 weeks	liver	0.06-0.23 vc	Southern blot					Values determined from figure
Ohashi et al., 2005	AAV2	rat (nude and Lewis)	male	hepatic artery infusion with selective liver lobes infusion	$5x10^{12}$ vg/kg	7 weeks	non-infused liver lobes infused liver lobes	0.008-0.01 vc 0.13-0.32 vc	Southern blot					Values determined from figure
Ohashi et al., 2005	AAV2	rat (nude and Lewis)	male	portal vein infusion	$5x10^{12}$ vg/kg	7 weeks	liver	0.01-0.04 vc	Southern blot					Values determined from figure
Grimm et al., 2003	AAV2	mice (C57BL/6)	? (n = 3)	intra-venous (portal vein)	$2x10^{11}$ gc	6 weeks	liver heart kidney spleen brain lung	+ + + + + +	Q-PCR and Southern blot					ratio portal/tail vein measured with Q-PCR: is 9.1

Nathwani et al., 2002	AAV2 (CAGG-hFIX)	monkey (Macaque)	male (n=5)	intra-arterial (hepatic artery)	$4x10^{12}$ vp/kg	11 months	3 days 6 days 8 days 11 months	plasma liver spleen kidneys gonads	+ (4/4) + (1/4) - + + - -	PCR	3 days 6 days 8 days 3 days 6 days 8 days	saliva urine	+ (1/4) - - + (1/4) + (1/4) -	PCR
Nathwani et al., 2001	AAV2 (rAAV CAGG-FIX)	monkey (Macaque)	male	intra-venous (tail vein)	$5 * 10^{10}$ vg		22 weeks	liver lung spleen kidney heart	+ (0.32 vc) - - - -	Q-PCR				
Nathwani et al., 2001	AAV2 (rAAV CAGG-FIX)	monkey (Macaque)	male	intra-venous (portal vein)	$1 * 10^{11}$ vg		22 weeks	liver lung spleen kidney heart	+ (0.5 vc) - + - +					
Arruda et al., 2001	AAV2	rat	? (n = 12)	intra-venous (into hepatic artery)	$1x10^{11}$ vg/kg		50 days 92 days 50 days 92 days	gonads gonads gonads gonads	1/6 0/6 2 -	PCR vector copy/µg DNA				
Arruda et al., 2001	AAV2	rat	? (n = 11)	intra-venous (into hepatic artery)	$1x10^{12}$ vg/kg		50 days 92 days 50 days 92 days	gonads gonads gonads gonads	4/6 0/5 1-3 -	PCR vector copy/µg DNA				
Arruda et al., 2001	AAV2	rat	? (n = 12)	intra-venous (into hepatic artery)	$1x10^{13}$ vg/kg		50 days 92 days 50 days 92 days	gonads gonads gonads gonads	6/6 4/6 4-16 2-7	PCR vector copy/µg DNA				
Arruda et al., 2001	AAV2	dog	? (n = 3)	intra-venous (into hepatic artery)	$3.7x10^{12}$ – $7x10^{12}$ vg/kg		90 days	gonads	0/3	PCR	7 days 30 days 60 days 90 days 90 days	semen semen	0/3 0/3 0/3 0/3 -	PCR vector copy/µg DNA
Snyder et al., 1999	AAV2 (MFGh FIX)	mice (C57Bl/6x hemophilia B)		intra-portal	$2.1*10^{10}$ to $8.4*10^{10}$ vp	36 weeks	1 day 3 days 7 days 35 days 91 days 1 day 3 days 7 days 35 days 91 days 1 day 3 days 7 days 35 days 91 days	liver muscle spleen 	+ + + + - + - - - - + + - - -	Southern blot				

Snyder et al., 1997	AAV2 (MFGh TH)	mice (C57Bl/6)		intra-portal	2.1*10^{10} to 8.4*10^{10} vp	36 weeks	7 weeks	liver	1.5 and 3.7 vc (up to 4.2*10^{10})	Southern blot					
Nathwani et al, 2007	scAAV2/5 (LP1-hFICco)	monkey (Macaca mulatta) (3-6 years)	male	intra-venous (peripheral and mesenteric vein)	1x10^{12} vp/kg		0-7 days	plasma	+	Q-PCR					
								liver	+	RT-PCR					
								spleen	+						
								kidney	+						
								testis	+						
Nathwani et al, 2007	scAAV2/8 (LP1-hFICco)	monkey (Macaca mulatta) (3-6 years)	male	intra-venous (saphenous vein)	1x10^{12} vp/kg		0-72 h	plasma	+	Q-PCR	0-72 h	urine	-	semiQ-PCR	
							> 72 h	plasma	-			saliva	-		
							> 72 h	liver	+	semiQ PCR					
								spleen	+						
								kidney	+						
								testis	+						
Mori et al., 2006	AAV2/10 (EGFPtub)	monkey (Cynomolgus)	male (n = 1) and female (n = 2)	intra-venous	1.0x10^{10} gc/kg	7 months	3 months	cerebrum	-	PCR					
							7 months	cerebellum	-						
								bone marrow	-						
								skin	+ (1/3)						
								retina	-						
								muscle	-						
								trachea	-						
								lung	-						
								heart	-						
								liver	+ (1/3)						
								gallbladder	-						
								pancreas	-						
								spleen	+++ (3/3)						
								oesophagus	-						
								stomach	-						
								jejunum	-						
								ileum	+ (1/3)						
								colon	+ (1/3)						
								kidney	-						
								adrenal gland	-						
								bladder	++ (1/3)						
								tonsil	++ (2/3)						
								thymus	-						
								parotid gland	+ (2/3)						
								submandibular gland	+++ (1/3)						
								thyroid gland	-						
								axillary lymph node	++ (3/3)						
								hilar lymph node	++ (3/3)						
								mesenteric lymph node	+ (3/3)						
								iliac lymph node	++ (2/3)						
								inguinal lymph node	++ (3/3)						
								testis/ovary	-						
								epididymis/uterus	-						

Mori et al., 2006	AAV2/11 (EGFPtub)	monkey (Cynomolgus)	male (n = 1) and female (n = 2)	intra-venous	$1.0x10^{10}$ gc/kg	7 months	3 months (n = 2) 7 months (n= 1)	cerebrum	-	PCR
								cerebellum	-	
								bone marrow	-	
								skin	-	
								retina	-	
								muscle	-	
								trachea	-	
								lung	-	
								heart	-	
								liver	-	
								gallbladder	-	
								pancreas	-	
								spleen	++ (2/3)	
								oesophagus	-	
								stomach	-	
								jejunum	-	
								ileum	-	
								colon	-	
								kidney	-	
								adrenal gland	-	
								bladder	+++ (1/3)	
								tonsil	++ (1/3)	
								thymus	-	
								parotid gland	-	
								submandibular gland	-	
								thyroid gland	-	
								axillary lymph node	+ (2/3)	
								hilar lymph node	+ (1/3)	
								mesenteric lymph node	+ (1/3)	
								iliac lymph node	+ (1/3)	
								inguinal lymph node	++ (1/3)	
								testis/ovary	-	
								epididymis/uterus	-	
Grimm et al., 2003	AAV3	mice (C57BL/6)	? (n = 3)	intra-venous (portal vein)	$2x10^{11}$ gc	6 weeks	liver	+	Q-PCR and Southern blot	ratio/tail vein measured with Q-PCR: is 48
							heart	+		
							kidney	+		
							spleen	+		
							brain	+		
							lung	+		
Grimm et al., 2003	AAV4	mice (C57BL/6)	? (n = 3)	intra-venous (portal vein)	$2x10^{11}$ gc	6 weeks	liver	+	Q-PCR and Southern blot	ratio portal/tail vein measured with Q-PCR: is 1
							heart	+		
							kidney	+		
							spleen	+		
							brain	+		
							lung	+		
Grimm et al., 2003	AAV5	mice (C57BL/6)	? (n = 3)	intra-venous (portal vein)	$2x10^{11}$ gc	6 weeks	liver	+	Q-PCR and Southern blot	ratio portal/tail vein measured with Q-PCR: is 15
							heart	+		
							kidney	+		
							spleen	+		
							brain	+		
							lung	+		
Grimm et al., 2003	AAV6	mice (C57BL/6)	? (n = 3)	intra-venous (portal vein)	$2x10^{11}$ gc	6 weeks	liver	+	Q-PCR and Southern blot	ratio portal/tail vein measured with Q-PCR: is 8.9
							heart	+		
							kidney	+		
							spleen	+		
							brain	+		
							lung	+		

Table B.13. Intraperitoneal administration of AAV

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	result	analysis method	time point	tested compartments	result	analysis technique		
Wang et al., 2005b	AAV1	neonatal mice (C57BL10)	male and female	intra-peritoneal	$2x10^{11}$ vp		2 months	abdominal tissue	11	Southern blot						
								diaphragm	6							
								intercostal	2							
								triceps	<0.1							
								masseter	<0.1							
								heart	<0.1							
								liver	<0.1							
								lung	<0.1							
								kidney	<0.1							
								spleen	<0.1							
Wang et al., 2005b	AAV2	neonatal mice (C57BL10)	male and female	intra-peritoneal	$2x10^{11}$ vp		2 months	abdominal tissue	6	Southern blot						
								diaphragm	3							
								intercostal	0.4							
								triceps	<0.1							
								masseter	<0.1							
								heart	<0.1							
								liver	<0.1							
								lung	<0.1							
								kidney	<0.1							
								spleen	<0.1							
Ogura et al., 2006	AAV5 (CMV-Luc)	neonate mice (C57BL/6)	male	intra-peritoneal	$1x10^{10}$ gc/g	16 weeks	10 weeks	brain	-	Q-PCR						
								lung	+							
								heart	+							
								liver	+							
								spleen	+							
								peritoneum	+							
								kidney	+							
								testis	-							
Ogura et al., 2006	AAV5 (CMV-Luc)	neonate mice (C57BL/6)	female	intra-peritoneal	$1x10^{10}$ gc/g	16 weeks	10 weeks	brain	-	Q-PCR						
								lung	+							
								heart	+							
								liver	+							
								spleen	+							
								peritoneum	+							
								kidney	+							
								ovaries	+							
Ogura et al., 2006	AAV5 (CMV-Luc)	adult mice (C57BL/6)	male	intra-peritoneal	$1x10^{10}$ gc/g	16 weeks	10 weeks	brain	-	Q-PCR						
								lung	+							
								heart	+							
								liver	+							
								spleen	+							
								peritoneum	+							
								kidney	+							
								testis	-							
Ogura et al., 2006	AAV5 (CMV-Luc)	adult mice (C57BL/6)	female	intra-peritoneal	$1x10^{10}$ gc/g	16 weeks	10 weeks	brain	-	Q-PCR						
								lung	+							
								heart	+							
								liver	+							
								spleen	+							
								peritoneum	+							
								kidney	+							
								ovaries	+							

Wang et al., 2005b	AAV8	neonatal mice (C57BL10)	male and female	intra-peritoneal	$2x10^{11}$ vp	2 months	abdominal tissue diaphragm intercostal triceps masseter heart liver lung kidney spleen testis	8 10 6 4 2 4 0.8 0.6 0.3 <0.1 <0.1	Southern blot	
Wang et al., 2005b	AAV1 AAV2 AAV5 AAV6 AAV7 AAV8	neonatal mice (C57BL10)	male and female	intra-peritoneal	$2x10^{11}$ vp of each vector in different animals	2 months	strong and widespread GFP expression in chest and hind leg muscles after AAV1, 6, 7 and 8 vector treatment, and more widespread expression in the forelimb and shoulder muscles after AAV7 and 8 treatment. Lower levels of fluorescence were observed in the AAV5 and AAV2 treated mice, mainly in abdominal muscle localized on the vector injection site.		whole-body fluorescent photograph y	The results indicate that AAV1, 6, 7 and most particularly AAV8 are highly efficient in transducing muscles beyond the intra-peritoneal cavity.

Table B.14. Intra-muscular administration of AAV

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	result	analysis method	time point	tested compartments	result	analysis technique		
Cao et al., 2007	AAV1 (hFIX)	mice (FIX ko C57BL/6)	?	intra-muscular (hind limbs)	$5x10^{10}$ gc	6 weeks	6 weeks	hind leg muscle abdominal muscle liver heart kidney	+ - - - -	immuno-histology						
Arruda et al., 2004	AAV1	mice (immunodeficient CD4 ko C57BL/6)	?	intra-muscular (tibialis enterior and quadriceps muscle)	$2x10^{11}$ $1.2x10^{12}$ $4x10^{12}$ vg/kg		12 weeks	injected muscle	14.5 gc 20.0 gc 33.0 gc	Southern blot						
Arruda et al., 2005	AAV2 (cFIX)	dog	female (n = 1)	isolated limb perfusion and intra-muscular	$2x10^{12}$ vg/kg		1 -5 days > 5 days 8 weeks	serum serum liver lung kidney spleen ovary contralateral muscle perfused skeletal muscle	+ - - - - - - - +	PCR						
Arruda et al., 2004	AAV2	mice (immunodeficient CD4 ko C57BL/6)	?	intra-muscular (tibialis enterior and quadriceps muscle)	$2x10^{11}$ vg/kg		12 weeks	injected muscle	0.4 gc	Southern blot						
Arruda et al., 2004	AAV2	mice (immunodeficient CD4 ko C57BL/6)	?	intra-muscular (tibialis enterior and quadriceps muscle)	$1.2x10^{12}$ vg/kg		12 weeks	injected muscle	3.0 gc	Southern blot						
Arruda et al., 2004	AAV2	mice (immunodeficient CD4 ko C57BL/6)	?	intra-muscular (tibialis enterior and quadriceps muscle)	$4x10^{12}$ vg/kg		12 weeks	injected muscle	15.0 gc	Southern blot						
Pachori et al., 2004	AAV2 (LacZ)	rat (Sprague-Dawley)	male (n = 4)	intra-myocardial	$4x10^{11}$ vp		6 months	testes heart kidney liver spleen	+ + + + -	RT-PCR						no positive spermatids or mature sperm cells were detected
Manno et al., 2003	AAV2 (hFIX)	human (haemophilia patients)	male (n = 8)	intra-muscular (leg)	$1.4x10^{13}$ – $7.0x10^{14}$ vg	24 weeks	0 days 1 day 2 days 4 days 6 days 7 days 2 weeks 3 weeks 4 weeks 5 weeks 6 weeks 7 weeks 8 weeks 10 weeks 12 weeks 14 weeks	serum	1/4 6/6 5/6 1/2 0/1 4/8 0/4 0/4 1/4 0/1 0/4 1/2 0/5 0/2 1/4 0/1		0 days 1 days 2 days 3 days 4 days 7 days 2 weeks 3 weeks 4 weeks 5 weeks 6 weeks 7 weeks 8 weeks 10 weeks 12 weeks	saliva	2/4 6/6 2/6 0/2 0/1 0/7 1/4 0/4 0/4 0/1 0/3 0/3 0/3 0/1 0/2		

							16 weeks		0/1		0 days	urine	1/4			
											1 day		1/6			
											2 days		0/5			
											3 days		0/1			
											4 days		0/1			
											6 days		0/1			
											7 days		0/7			
											2 weeks		0/6			
											3 weeks		0/6			
											4 weeks		0/6			
											5 weeks		0/1			
											6 weeks		0/2			
											7 weeks		0/3			
											8 weeks		0/4			
											10 weeks		0/1			
											12 weeks		0/3			
											2 days	semen	0/1			
											3 days		0/2			
											4 days		0/2			
											7 days		0/5			
											2 weeks		0/2			
											3 weeks		0/2			
											4 weeks		0/2			
											5 weeks		0/1			
											6 weeks		0/1			
											7 weeks		0/2			
											8 weeks		0/2			
											24 weeks		0/1			
Song et al., 2002	AAV2	monkey (Baboon)	?	intra-muscular	5 * 10^{12} vg/kg	4 months	4 months	gonads	-	Q-PCR						
Gao et al., 2002	AAV2	mice (C57BL/6)	? (n=3)	intra-muscular	1x10^{11} gc		56 days	liver	0.003±0.001							
Nathwani et al., 2001	AAV2 (hFIX, several promoters)	mice (BalB/C, Fv129, C57Bl/6, and SCID mice (C.B-17 SCID))	male	intra-muscular	5 * 10^{10} vg		22 weeks	liver lung spleen kidney heart	+ (0.2 vc) + + + +	PCR						rAAV-mediated expression highest after injection in portal vein. Expression is promoter specific
Favre et al., 2001	AAV2 (cmEPO , with two different promoters)	monkey (Rhesus and Cynomolgus)	male (n = 6) and female (n = 2)	intra-muscular (tibialis anterior, gastrocne-mius, quadriceps, soleus) (n=8)	5x 10^{8}-1x10^{10} ip/kg	18 months	30 min 6 h 1 day 2 days 3-6 days 7 days	serum	5/8 4/4 8/8 8/8 5/8 0/8	PCR	6 h 1 day 2 days 3-6 days 7 days	urine	2/4 7/8 4/8 2/8 0/8	PCR		
							30 min 6 h 24 h 48 h >7 days	serum	5/8 (<800 ip/ml) 4/4 (<475 ip/ml) 8/8 (<600 ip/ml) 7/8 (<100 ip/ml)	mRCA	1 day 2 day 3-6 days 7 days 1 days 2 days	faeces saliva	4/8 3/8 2/8 0/8 3/8 2/8			
							8, 12 or 18 months	muscle liver lymph node lung spleen kidney heart brain gonads	6/6 6/6 4/4 0/4 0/4 0/4 0/4 0/2 0/6	PCR	3-6 days 7 days 6 h 1 days 2 days 3-6 days 7 days	lacrymal	0/8 0/8 1/4 2/8 3/8 2/8 0/8			

							intestine	0/4		6 h 1 days 2 days 3-6 days 7 days	nasal	2/4 3/6 5/6 4/8 0/8			
Arruda et al., 2001	AAV2	mice	? (n = 10)	intra-muscular	1.7x10^{11} vg/kg	31 days 91 days 31 days 91 days	gonads gonads gonads gonads	1/5 2/5 10-100 1-10	PCR vector copy/µg DNA							
Arruda et al., 2001	AAV2	mice	? (n = 8)	intra-muscular	1.7x10^{12} vg/kg	31 days 91 days 31 days 91 days	gonads gonads gonads gonads	3/3 3/5 10-100 10-100 (2/3) >100 (1/3)	PCR vector copy/µg DNA							
Arruda et al., 2001	AAV2	rat	? (n = 5)	intra-muscular	2.8x10^{11} vg/kg	15 days 15 days	epididymal effluent epididymal effluent	2/5 1-10	PCR vector copy/µg DNA							
Arruda et al., 2001	AAV2	rat	? (n = 4)	intra-muscular	2.8x10^{13} vg/kg	15 days 15 days	epididymal effluent epididymal effluent	3/4 1-10	PCR vector copy/µg DNA							
Arruda et al., 2001	AAV2	rabbit	male (n = 12)	intra-muscular	1x10^{13} vg/kg	15 min 24 h 48 h 7 days 7 days 30 days 60 days 90 days	serum gonads 	+ + + - 3/3 2/3 2/3 1/3	PCR PCR 	7 days 30 days 60 days 90 days	semen	- - - -	PCR		hematogenous dissemination and binding to HSPG on testis blood vessel	
Arruda et al., 2001	AAV2	dog	? (n = 4)	intra-muscular	1.3x10^{11}-1.1x10^{13} vg/kg					1.5 month 4 months 14 months 16 months 1.5 month 4 months 14 months 16 months	semen semen 	0/4 0/4 0/4 0/4 - - - -	PCR vector copy/µg DNA 			
Kay et al., 2000	AAV2 (CMV-hF.IX)	human (haemophilia B patients)	male (n = 3)	in vastus lateralis (10-12x)	2 * 10^{11} vg/kg	ongoing	? (24h-1y) 2, 6 and 12 months	serum muscle	+ at 24 and 48 h +	PCR	24 h 48 h 4 days8 5 days6 5 days9 24 h 48 h 4 days8 5 days6 5 days9 24 h to 59 days 24 h to 59 days	urine saliva stool	+ (1) – - - - + – - - - - -	PCR PCR PCR 	no clear indication at which time points serum levels and shedding was measured	

Chao et al., 1999	AAV2 (cFIX)	dog (Chapel Hill)	female (n = 1)	intra-muscular (all leg)	$1.2x10^{13}$ vp	33 weeks	33 weeks	blood, pMNC	-	PCR
								brain	-	
								gonads	-	
								heart	-	
								intestine	-	
								kidney	-	
								liver	-	
								lung	-	
								lymph nodes	-	
								muscle, control	-	
								muscle, injected	+	
								spinal cord	-	
								spleen	-	
								blood, pMNC	-	RT-PCR
								brain	-	
								gonads	-	
								heart	-	
								intestine	-	
								kidney	-	
								liver	+	
								lung	-	
								lymph nodes	-	
								muscle, control	-	
								muscle, injected	+	
								spinal cord	-	
								spleen	-	
Monahan et al., 1998	AAV2 (hFIX)	dog (Chapel Hill)	male (n = 1)	intra-muscular (hind legs)	$2.5x10^{12}$ vp		10 weeks	adrenal gland	-	PCR
								brain	-	
								kidney	-	
								liver	-	
								lung	-	
								lymph node, right inguinal	+	
								muscle, control	-	
								muscle, injected	+	
								testis	-	
								thymus	-	
								thyroid	-	

Gao et al., 2002	AAV2/5	mice (C57BL/6)	? (n=3)	intra-muscular	$1x10^{11}$ gc		56 days	liver	0.83±0.64	
Gao et al., 2002	AAV2/7	mice (C57BL/6)	? (n=3)	intra-muscular	$1x10^{11}$ gc		56 days	liver	2.2±1.7	
Gao et al., 2002	AAV2/8	mice (C57BL/6)	? (n=3)	intra-muscular	$1x10^{11}$ gc		56 days	liver	18±11	
Cao et al., 2007	rAAV8 (hFIX)	mice (FIX ko C57BL/6)	?	intra-muscular (hind limbs)	$5x10^{10}$ and $2.5x10^{12}$ gc	6 weeks	6 weeks	hind leg muscle abdominal muscle liver heart kidney	+ - - - -	immuno-histology

Table B.15. Inhalatory and intranasal-bronchial administration of AAV

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution				Shedding				Kinetic parameters	Notes
							time point	tested organs	result	analysis method	time point	tested compartments	result	analysis technique		
Moss et al., 2004	AAV2 (tgCFTR)	human (CF patients)	male and female (n = 37)	inhalation	1x 10^{13} drp	150 days					0 days	sputum	90% ($6.79*10^5$ i.u./ml)	colony forming units		
											14 days		5% (<4000 i.u./ml)			
											60 days		35% (<4000 i.u./ml)			
											75 days		13% (<6000 i.u./ml)			
											90 days		0%			
											150 days		18% (<4000 i.u./ml)			
Flotte et al., 2003	AAV2 (tgAAVCF)	human (CF patients)	male and female (n = 25)	nasal and bronchial	$3x10^1$ - $1x10^9$ ru	24 months					1 day	sputum	+ (1/25)	non-Q PCR		• first 16 subject could not be tested for shedding due to assay difficulties • 7 days stated in table 3B and 3 days0 stated in text, not clear which time point was used
											7 /30 days	sputum	-			
											1 – 30 days	BAL	-			
												nasal washings	-			
												urine	-			
												stool	-			
Aitken et al., 2001	AAV2	human (CF patients)	male and female (n = 12)	inhalatory	10^{10} - 10^{13} drp escalating in 10-fold increments		1 day	blood	+ (1/12) ~ (2/12) - (9/12)	PCR	1-90 days	stool urine	- -	culture		
											1 days 7 days 14 days 30 days	sputum	7/12 1/2 0/3 0/2			
Wagner et al., 1999	AAV2 (CFTR)	human (CF patients)	male (n = 5) and female (n = 5)	maxillary sinus	$1 * 10^2$ – $1 * 10^5$ ru	34 days	1 day and 7 days	blood	-	semiQ-PCR	2-34 days	nose secretion	-	SQ-PCR		
												stool	-			
												urine	-			

Table B.16. Ocular administration of AAV

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution			Shedding			Kinetic parameters	Notes
							time point	tested organs	result	analysis method	time point	tested compartments	result	analysis technique		
Jacobson et al., 2006b	AAV2 (RPE65)	monkey (Cynomolgus)	male (n = 2) and female (n = 2)	intra-ocular	$1.5x10^{12}$ – $3.3x10^{12}$ vg		1 week	heart	-	Q-PCR						
								lung	-							
								liver	-							
								pancreas	-							
								spleen	-							
								kidney	-							
								jejunum	-							
								gonads	-							
								skeletal muscle	-							
								preauricular lymph node	+ (2/4)							
								mandibular lymph node	+ (1/3)							
								tracheobronchial lymph node	-							
								mesenteric lymph node	-							
								left vitreous	-							
								left retina	-							
								left optic nerve	-							
								left orbital tissue	-							
								right vitreous	+ (3/3)							
								right retina	+ (4/4)							
								right optic nerve	+ (4/4)							
								right orbital tissue	-							
								optic chiasm	-							
								left optic tract	+ (2/4)							
								left LGN	-							
								left visual cortex	+ (1/4)							
								left superior colliculus	+ (1/4)							
								right optic tract	+ (3/4)							
								right LGN	+ (2/4)							
								right visual cortex	-							
								right superior colliculus	-							
								left thalamus	-							
								right thalamus	-							
								cerebellum	-							
Jacobson et al., 2006b	AAV2 (RPE65)	monkey (Cynomolgus)	male (n = 3) and female (n = 3)	intra-ocular	$1.5x10^{12}$ – $4.5x10^{12}$vg		3 months	heart	-							
								lung	-							
								liver	-							
								pancreas	-							
								spleen	-							
								kidney	-							
								jejunum	-							
								gonads	-							
								skeletal muscle	-							
								preauricular lymph node	-							
								mandibular lymph node	-							
								tracheobronchial lymph node	-							
								mesenteric lymph node	-							
								left optic nerve	-							
								left orbital tissue	-							
								right optic nerve	-							
								right orbital tissue	-							
								optic chiasm	-							
								left optic tract	-							
								left LGN	+ (1/6)							
								left optic radiation	-							

								left visual cortex	-				
								left superior colliculus	-				
								right optic tract	-				
								right LGN	-				
								right optic radiation	-				
								right visual cortex	-				
								right superior colliculus	-				
								left thalamus	-				
								right thalamus	-				
								cerebellum	-				
Jacobson et al. 2006a	AAV2	dog (RPE-mutant)	male + female (n = 13)	ocular	$1.5x10^{8}$-$4.5x10^{12}$ vg		3 months	left optic nerve	1/11	real-time PCR			
								right optic nerve	0/11				
								chiasm	1/12				
								optic tract	0/4				
								lateral geniculate	0/5				
								nucleus	0/4				
								visual cortex	0/2				
								superior colliculus	0/11				
								left periocular	0/10				
								right periocular	1/6				
								left mandibular node	1/8				
								right mandibular node	0/10				
								parotid node	1/12				
								left heart	0/10				
								right heart	0/10				
								lung	2/9				
								diaphragm	0/6				
								left liver	0/6				
								right liver	0/12				
								pancreas	0/8				
								spleen	0/13				
								left kidney	0/11				
								right kidney	0/9				
								jejunum	0/9				
								left gonads	0/12				
								right gonads	0/12				
								skeletal muscle					
Weber et al., 2003	AAV2/4 (CMV.gfp)	monkey (Cynomolgus)	(n = 2)	intra-ocular (subretinal)	$2.4x10^{11}$ – $4x10^{11}$ vg	2 months	2 months	serum (n = 1)	+ 2 h-14 d	PCR	2 months	nasal fluid (n=1)	+ 15 min–2h
								lachrymal (n=1)	+ 15 min – 2 h			urine	-
												faeces	-
Weber et al., 2003	AAV2/4 (CMV.gfp)	dog (Beagle)	(n = 3)	intra-ocular (subretinal)	$2.4x10^{11}$ – $4x10^{11}$ vg	2 months	2 months	serum	+ 15 min – 25 d	PCR	2 months	nasal fluid	15 min – 1 d
								lacrymal	+ 15 min – 3 d				
Weber et al., 2003	AAV2/5 (CMV.gfp)	dog (Beagle)	(n = 2)	intra-ocular (subretinal)	$1.2x10^{11}$ – $2x10^{11}$ vg	2 months	2 months	serum	+ 2 d – 23 d	PCR	2 months	nasal fluid	15 min – 4 d
								lacrymal	+ 15 min – 3 d				

Table B.17. Other administration routes of AAV

Reference	Vector	Species/ subspecies	Gender	Route	Dose	Duration of study	Biodistribution			Shedding				Kinetic parameters	Notes
							time point	tested organs	results	analysis method	time point	tested compartments	results	analysis method		
Voutetakis et al., 2007	AAV2 (rhEPO)	monkey (Macaque)	male (n=3)	oral cannulation of right parotid gland	$1x10^{10}$ vp	6 months	6 months	left gonad	≤ 60	Q-PCR						
								right gonad	≤ 60							
								lung	≤ 60							
								heart	≤ 130							
								left kidney	≤ 60							
								right kidney	≤ 60							
								spleen	≤ 60							
								liver	≤ 60							
								left SMG	≤ 60							
								right SMG	≤ 60							
								left CLN	≤ 50							
								right CLN	≤ 50							
								left parotid	≤ 60							
								right parotid (superior)	90 ± 10							
								right parotid (middle)	150 ± 30							
								right parotid (inferior)	140 ± 50							
Voutetakis et al., 2007	AAV2 (rhEPO)	monkey (Macaque)	male (n=3)	oral cannulation of right parotid gland	$1x10^{11}$ vp	6 months	6 months	left gonad	≤ 70	Q-PCR						
								right gonad	≤ 70							
								lung	≤ 70							
								heart	≤ 130							
								left kidney	≤ 70							
								right kidney	≤ 70							
								spleen	≤ 70							
								liver	≤ 70							
								left SMG	≤ 70							
								right SMG	≤ 70							
								left CLN	≤ 80							
								right CLN	≤ 80							
								left parotid	≤ 60							
								right parotid (superior)	9450 ± 3680							
								right parotid (middle)	9520 ± 28220							
								right parotid (inferior)	14280 ± 8810							
Voutetakis et al., 2004	AAV2 (hEPO or LacZ)	mice (BALB/C)	male (n = 4)	retrograde ductal delivery to submandibul ar glands	10^9 vp		8 weeks	salivary glands	13185 vc	Q-PCR						
								liver	567 vc							
								spleen	336 vc							
								testes	183 vc							
Voutetakis et al., 2004	AAV2 (hEPO or LacZ)	mice (naïve)	male (n = 4)	retrograde ductal delivery to submandibul ar glands	10^9 vp		8 weeks	salivary glands	480 vc	Q-PCR						
								liver	511 vc							
								spleen	303 vc							
								testes	204 vc							
Arruda et al., 2005	AAV2 (cFIX)	dog	female (n = 1)	isolated limb perfusion and intra-muscular	$2x10^{12}$ vg/kg		1 – 5 days	serum	+	PCR						
							> 5 days	serum	-							
							8 weeks	liver	-							
								lung	-							
								kidney	-							
								spleen	-							
								ovary	-							
								contralateral muscle	-							
								perfused skeletal muscle	+							

Arruda et al., 2005	AAV2 (cFIX)	dog	female (n = 1)	isolated limb perfusion and intra-muscular	$2x10^{12}$ vg/kg		1 – 5 days > 5 days 8 weeks	serum serum liver lung kidney spleen ovary contralateral muscle perfused skeletal muscle	+ - - - - - - - +	PCR	
Passini et al., 2001	AAV2	neonatal mice (C3H/HeOuJ)		intraventricular (cerebral lateral ventricle)	$1.8x10^{10}$ vp		30 min 20 h 1 week 1 month 6 months 12 months	forebrain, caudal forebrain, rostral hindbrain midbrain forebrain, caudal forebrain, rostral hindbrain midbrain pial surface brain parenchyma meninges brain	+ ++ + + ++ + ++ ++ + + + + + +	fluorescent analysis ISH ISH	
Ulrich-Vinther et al., 2004	AAV2 (eGFP)	rabbit (New Zealand White)	female (n = 6)	intra-articular	$1.5x10^{12}$ ip	21 days	2 days1	serum	-	titer assay (infectious particles)	
Kho et al., 2000	AAV2 (β-gal or GFP/NT3 myc)	guinea pigs (Hartley)	male	intra-cochlear	$5*10^{8}$ vp	8 weeks	2 and 4 weeks	injected cochlea contralateral cochlea cerebellum cortex heart lung liver spleen kidney	+ (8/8) + (8/8) + (8/8) - - - - - -	PCR	
Cearley and Wolfe, 2007	AAV9 (hGUSB)	mice (C3H/HeOuJ) (normal and MPS VII-affected)	?	brain (hippocampus)	$1.2x10^{10}$ – $1.3x10^{10}$ gc		1 h 24 h	brain	injection site injection site dentate gyrus pyramidal cell layer oriens cell layer retrospenial cortex entorhinal cortex	immuno-histology	distribution throughout the brain to the: • prefrontal cortex • septal nuclei • habenula • thalamus • locus ceruleus • tegmental nuclei • cerebellar nuclei • piriform cortex • striatum • amygdala

Cearley and Wolfe, 2007	AAV9 (hGUSB)	mice (C3H/HeOuJ) (normal and MPS VII-affected)	?	brain (VTA)	$1.2x10^{10}$ – $1.3x10^{10}$ gc	?	brain	retrospenial cortex striatum habenula thalamus endopiri-form nuclei	immuno-histology			distribution throughout the brain to the: • prefrontal cortex • septal nuclei • habenula • thalamus • locus ceruleus • tegmental nuclei • cerebellar nuclei • piriform cortex • striatum amygdala
Mori et al., 2007	AAV10	monkey (Cynomolgus)	male (n = 5) and female (n = 9)	naturally infected (probably inhalatory)	?		cerebrum cerebellum spinal cord bone marrow skin eye muscle bronchus lung heart liver gallbladder pancreas spleen oesophagus stomach jejunum ileum colon kidney adrenal glad bladder tonsil thymus parotid gland submandibular gland thyroid gland axillary lymph node hilar lymph node mesenteric lymph node iliac lymph node inguinal lymph node testis/ovary epididymis/uterus	0/14 0/14 0/11 2/14 0/13 0/14 0/13 0/14 0/14 1/14 1/14 0/14 1/14 4/14 0/14 1/14 1/12 2/14 0/13 1/14 2/14 0/13 0/14 0/12 0/14 0/14 0/14 3/14 0/8 1/13 0/10 3/14 0/14 0/13	PCR			11 monkeys were infected with 2 or 3 types of AAV

Mori et al., 2007	AAV11	monkey (Cynomolgus)	male (n = 5) and female (n = 9)	naturally infected (probably inhalatory)	?	cerebrum	1/14	PCR
						cerebellum	0/14	
						spinal cord	1/11	
						bone marrow	1/14	
						skin	0/13	
						eye	1/14	
						muscle	0/13	
						bronchus	0/14	
						lung	1/14	
						heart	2/14	
						liver	2/14	
						gallbladder	2/14	
						pancreas	0/14	
						spleen	4/14	
						oesophagus	1/14	
						stomach	1/14	
						jejunum	0/12	
						ileum	2/14	
						colon	1/13	
						kidney	1/14	
						adrenal glad	2/14	
						bladder	0/13	
						tonsil	2/14	
						thymus	0/12	
						parotid gland	0/14	
						submandibular gland	0/14	
						thyroid gland	1/14	
						axillary lymph node	2/14	
						hilar lymph node	2/8	
						mesenteric lymph node	1/13	
						iliac lymph node	2/10	
						inguinal lymph node	2/14	
						testis/ovary	0/14	
						epididymis/uterus	0/13	
Mori et al., 2007	AAVcy.7	monkey (Cynomolgus)	male (n = 5) and female (n = 9)	naturally infected (probably inhalatory)	?	cerebrum	3/14	PCR
						cerebellum	0/14	
						spinal cord	2/11	
						bone marrow	1/14	
						skin	0/13	
						eye	2/14	
						muscle	1/13	
						bronchus	0/14	
						lung	0/14	
						heart	4/14	
						liver	1/14	
						gallbladder	1/14	
						pancreas	0/14	
						spleen	6/14	
						oesophagus	1/14	
						stomach	3/14	
						jejunum	2/12	
						ileum	6/14	
						colon	1/13	
						kidney	0/14	
						adrenal glad	0/14	
						bladder	0/13	
						tonsil	4/14	
						thymus	1/12	
						parotid gland	1/14	
						submandibular gland	0/14	

	thyroid gland	1/14
	axillary lymph node	4/14
	hilar lymph node	1/8
	mesenteric lymph node	4/13
	iliac lymph node	1/10
	inguinal lymph node	3/14
	testis/ovary	1/14
	epididymis/uterus	1/13

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