Position statement of the ZKBS
on risk assessment of human adeno-associated viruses
and its derived vectors

Adeno-associated viruses

Adeno-associated viruses (AAV) are ubiquitous in a variety of animals and humans, where the host range of the individual serotypes is very limited [1; 2]. As part of a subgroup of defective viruses (genus Dependoparvovirus), they belong to the family Parvoviridae [3].

AAV particles are not enveloped and are relatively stable in the environment even when they dry out. The single-stranded DNA genome consists of the two open reading frames rep and cap. The four rep-encoded nonstructural proteins Rep78, Rep68, Rep52, and Rep40 are important for virus replication, expression of structural proteins and integration into the genome of the host cell. The three capsid proteins Vp1, Vp2 and Vp3 are encoded by cap to form the icosahedral nucleocapsid. Hypervariable regions of the capsid proteins influence tissue specificity, which can be quite diverse for the different AAV types [4; 5].

One inverted terminal repeat (ITR) delimits the genome at the 5’ and 3’ ends, respectively. The ITRs contain the cis-active elements for the replication of the viral genome and its integration into the host genome as well as the packaging signal. For productive, lytic infection, AAV require helper functions provided by helper viruses (such as adenovirus, herpes simplex virus type I and type II, cytomegalovirus or human herpesvirus 6). Although a cell will still get infected by AAV in the absence of helper functions, the transferred AAV genome rests in the nucleus of the host cell (latent infection), mainly as an extra-chromosomal episome. However, it may also be integrated in the host genome [6; 7]. In humans, AAV is the only known viral genome specifically integrating into the genome of the host cell. This integration usually occurs in the AAVS1 locus of chromosome 19 [6]. The latent virus can be mobilized again through superinfection with adenoviruses or herpesviruses.

AAV are believed to be transmitted via respiratory and faecal-oral routes [7; 8]. The more than one hundred AAVs isolated from primates so far can be subdivided into 13 serotypes [5; 9].

Serotypes AAV-2, -3, -5, -6 and -9 were isolated from humans [5; 10; 12].

Serotype AAV-1, however, was isolated from both monkeys and humans [5].

Serotypes AAV-4, -7, -8, -10, -11 and -12 were isolated from monkeys [5; 11-13].

Depending on the age and geographical area, about 80% of people have antibodies to AAV [2]. They exhibit some cross-reactivity to other AAV serotypes [6]. Overall, seropositivity for AAV-1, -2 and -3 is described for 70% of the people studied [2; 14-16]. About 45% have antibodies against AAV-5 and -6 [2; 14]. The seroprevalence for AAV-8 and -9 is slightly lower (~ 38%) [2; 14-16]. The seroprevalence for AAV-7 is about 10% and for AAV-4 less than 2% [15; 17]. The seroprevalence for serotypes AAV-10, -11, -12 and -13 is unknown.

Despite the ubiquitous distribution of AAV and high infestation, no AAV-associated disease has been reported in humans or animals to date, suggesting that AAV are nonpathogenic [2; 6; 7]. Furthermore, a protective effect of AAV, for example against the development of tumours/cancers, is being discussed [18; 19].
In 2001, the AAV serotypes AAV-2, -3, and -5 were assigned by the ZKBS to risk group 1, while AAV serotypes AAV-1, -4 and -6 to -12 were assigned to risk group 2. Furthermore, isolate AAV-3b, derived from AAV-3, was classified as risk group 1. AAV-rh10 was isolated from rhesus monkey tissue in 2003 [4] and assigned to risk group 2. A later study showed that 59% of people have antibodies to AAVrh10 [20].

In TRBA 462 ‘Classification of viruses in risk groups’, AAV serotypes 2, 3 and 5 are classified as risk group 1. AAV serotypes 1, 4 and 6-11 are classified as risk group 2.

The Dutch Commissie Genetische Modificatie (COGEM) has recently classified serotypes AAV-10 to -12 and AAVrh10 as risk group 1 [21]. In addition, the COGEM recommended that all AAVs of the species adeno-associated dependoparvovirus A and B belong to risk group 1. This was justified with the ubiquitous spread, the dependence on a helper virus and the absence of any evidence of pathogenicity.

The US National Institutes of Health (NIH) has assigned all AAV serotypes as nonpathogenic to risk group 1 [22].

**Risk assessment:**

AAV are widespread in a variety of vertebrates, including humans because of their high infectivity. However, due to the high seroprevalence for different AAV in humans, it can be assumed that the infection is not associated with any pathogenicity. For some AAV serotypes, no natural human infections are described, and seroprevalence is low or unknown. Even for these AAV pathogenicity is very unlikely, but ultimately cannot be safely ruled out.

Based on the new findings since the ZKBS position statements on the classification of AAV in 2001 and 2005, downgrades of individual AAV serotypes are made.

According to § 5 (1) GenTSV (German Genetic Engineering Safety Regulations) in conjunction with the criteria in Annex I GenTSV, the ZKBS recommends the following classification of the various AAV serotypes as donor and recipient organisms for genetic engineering operations:

**AAV-1 to -3, AAV-3b, AAV-5, AAV-6, AAV-8, AAV-9 and AAV-rh10:** Risk group 1

**AAV-4, AAV-7, AAV-10 to -13:** Risk group 2

**Note:**

Upon presentation of appropriate data, downgrading of further AAV serotypes may possibly occur.

**AAV-derived vector systems**

Recombinant AAV vectors are now being tested in numerous clinical trials as therapeutic agents for a wide variety of diseases [5; 18]. First AAV-based medicinal products have already been approved in Europe (Glybera®, Luxturna™).

A conventional AAV vector system consists of two plasmids, mostly derived from pBR322, and a helper virus [6]. From AAV, the transfer plasmid carries only the ITRs that are upstream and downstream of the nucleic acid segment to be transferred. Only the nucleotide sequences of the reading frames rep and cap are present on the helper plasmid of AAV. There is no overlap of homologous AAV nucleotide sequences between transfer and helper plasmid; thus, a homologous recombination is not expected.
For the production of recombinant, AAV-derived vectors, host cells are co-transfected with transfer and helper plasmid and infected with a helper virus, as this provides the essential viral helper functions for the propagation of AAV.

In advanced AAV vector systems, the viral helper functions are provided on a third plasmid independently of the helper virus, so that infection with a replication-competent helper virus is no longer necessary [6]. This also prevents the production of replication-competent helper viruses. Of the adenoviruses, previously used as helper viruses, the proteins E1a, E1b, E2a, E4 and VA are necessary for the production of AAV particles. By using the 293T cell line, which provides the adenoviral E1 proteins, only the genes for adenoviral proteins E2a, E4 and VA are required on the helper plasmids in addition to the rep and cap nucleotide sequences [23].

Risk assessment:
1. Recombinant, AAV-derived vector particles which, apart from the ITR, contain no nucleic acid sequences from AAV and no hazard-potential nucleic acid segments are classified as risk group 1, even if they are pseudotyped. The classification does not depend on which AAV the ITR comes from. Genetic engineering operation with genetically modified organisms that fulfil the mentioned criteria must be assigned to containment level 1.

2. Cells or cell lines of risk group 1, which are transduced with the recombinant, AAV-derived vector particles mentioned under section 1, are classified as risk group 1. Genetic engineering operation with genetically modified organisms that fulfil the mentioned criteria must be assigned to containment level 1. For cells that release organisms of higher risk groups, the hazard potential of the organisms is fully included in the risk assessment.

Justification:
In numerous clinical studies with AAV vectors of different human serotypes, it has been shown that the vectors do not enter the germ line [24]. Excretion of AAV vectors through trial subjects, for example through urine and saliva, was dependent on the administration route and dose [24]. However, the excretion is minute and transient, which limits the spread of the infectious AAV vector particles [25].

Within the host cell, the transfer DNA is mainly extrachromosomal. The vectors are replication-defective, and apart from the AAV-ITRs, there are no other genes of AAV or helper viruses on the transfer vector. In addition, the vector-receiver systems described under section 2 correspond to the biological safety measures according to § 6 (4) and (5) GenTSV (German Genetic Engineering Safety Regulations).

Notes:
1. In case of contamination of the recombinant, AAV-derived vectors described under section 1 with helper viruses, the hazard potential of these viruses is fully included in the risk assessment.

2. If there is a possibility that overlapping AAV nucleic acid segments on the transfer and helper plasmid give rise to complete, possibly chimeric AAV, or if such AAVs are generated on purpose, the risk assessment must be based on the AAV from which the nucleic acid segments for the rep proteins originate.

Reference is made to the 'Recommendation of the ZKBS on adenoviral and AAV-derived replication-defective viral particles that transfer a nucleic acid segment with neoplastically transforming potential' (ref_6790-10-83; updated version as of December 2016).
References


