

Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors¹

Note 1: This document has been endorsed by Austria, Belgium, Croatia, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Luxembourg, the Netherlands, Portugal, Romania and Spain.

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¹ This document has not been adopted by the European Commission and, therefore, it does not contain the official position of the European Commission.

1. INTRODUCTION

Adeno-associated viral vectors can be used to produce medicinal products. These vectors are derived from the adeno-associated virus (hereafter “AAV”), a single stranded DNA virus which belongs to the Parvoviridae family.

AAVs are widely distributed among a great number of animals and humans; it is estimated that approximately 80% of the adult population is seropositive to at least one AAV serotype. Despite of the ubiquitous distribution of AAVs and high frequency of AAV immunity, AAVs have not been associated with any pathogenic disease in humans or animals.

AAV viruses are unable to replicate unless the cell is co-infected with a helper virus. Adenovirus, herpes simplex virus, pseudorabies virus and human papilloma virus are known to support wild-type AAV replication. In presence of a helper virus, AAV undergoes a productive infection characterized by genome replication, viral gene expression and virion production. In absence of a helper virus in the infected cell, the viral DNA may persist in the host cell nucleus in episomal form or may integrate into the host cell genome, resulting in a latent infection. A latent AAV infection can be reactivated by infection of the cell by a helper virus. Integration of AAV into the host cell DNA at preferred *loci*, such as integration into the AAVS1 site, is mediated by the AAV Rep protein.

AAVs can infect both dividing and non-dividing cells and can infect a wide range of cell types, albeit specific serotypes may be associated to more efficient tissue tropism.

Adeno-associated viral particles are non-enveloped and are highly stable in the environment even when desiccated.

Almost the whole genome of the wild-type virus is removed in vectors used in clinical practice, with the exception of the inverted terminal repeats. This typically leaves a packaging capacity of 4.4 to 4.7 kb albeit, with certain production strategies, the packaging capacity of the AAV vector could be increased up to maximum of 6 kb.

While there are a number of manufacturing strategies that can be used to produce AAV clinical vectors, the basic functional elements are:

- The AAV ITR’s flanking the ‘gene of interest’ (this construct contains the cis elements necessary for packaging and replication of the single stranded DNA genome).
- Genetic sequences (Rep and Cap) necessary for AAV replication and viral capsid proteins (generally provided in trans within a plasmid, baculoviral vector, or in a packaging cell line).
- Helper virus functions: several approaches are possible, from the earlier approach of co-infection of the helper virus, to co-transfection with a plasmid or co-transduction with baculovirus derived vectors encoding the helper genes.
- A cell line capable of supporting helper virus and AAV replication.

Having regard to the above, as well as the accumulated experience with the assessment of AAV clinical vectors, the assessment of applications for the conduct of clinical trials with AAV clinical vectors should be based on the elements outlined in the *common application form for investigational medicinal products for human use that contain or consist of AAV vectors*.

Clinical trials with AAV clinical vectors in accordance with the requirements in the ERA in Section 2 can be conducted under BSL-1.

2. SPECIFIC ERA

This ERA is only applicable to AAV clinical vectors if the applicant demonstrates absence of formation of replication competent virus and that the transgene is not harmful.

2.1. Identification and characterization of hazards.

Potential hazards to human health

- *Hazards associated with the release of replication competent AAVs:* There is a potential hazard that the clinical vector would acquire replication competence if there was recombination of the clinical vector in individuals simultaneously infected by a wild-type AAV virus and a helper virus, which was followed by shedding into the environment. The recombined product would be an AAV. Considering that there is no known pathology associated to AAVs and that no hazardous insert is present in the clinical vector, the hazards associated with the release of replication competent AAVs can be regarded as very low.
- *Hazards associated with the long-term persistence of the clinical vector within transduced cells (latent infection):* The clinical vector is administered to patients with a view to treat an underlying condition. The persistence of the clinical vector in cells of the treated patient is an expected feature of the vector and it does not constitute a hazard to human health. However, the unintended transfer of the clinical vector to individuals other than the clinical trial subject could give rise to a transduction with the AAV clinical vector, in which case the DNA of the delivered AAV clinical vector is expected to persist in transduced cells of the non-targeted recipient as stable episomes, or the AAV clinical vector can -in rare instances- integrate (*see point below*).

Considering that there is no known pathology associated to AAVs and that no hazardous insert is present in the clinical vector, the hazards associated with the long-term persistency of the clinical vector within transduced cells can be regarded as very low.

- *Hazards associated with insertional mutagenesis:* Certain wild-type AAVs can stably integrate at a specific locus of the host cell genome (AAVS1 in human chromosome 19 long arm); in case of integration, they remain non-pathogenic. In contrast, recombinant AAVs have lost the ability to integrate at specific sites in the host cell genome.

The non-site specific integration of the clinical vector into the genome of the infected cells could lead to insertional mutagenesis in individuals that are exposed to the product by accident or *via* the environment (shedding).² If the risk of non-site integration would materialise, the consequences for the affected individuals could be regarded as moderate.

- *Hazards associated to vertical/germline transmission:* Biodistribution studies in animals have shown that DNA from AAV clinical vectors can be detected in gonadal DNA for a variable duration. Accordingly, the presence of recombinant AAV in the gonads cannot be excluded.

Restrictions exist under EU framework regulating the conduct of clinical trials with medicinal products for human use that prevent the occurrence of hazards associated to vertical/germline transmission (prohibition of modification of human germline and restrictions on the conduct of clinical trials with pregnant and breastfeeding women). Accordingly, this scenario is not further addressed in this ERA.

Potential hazards to animals or the environment

- *Hazards to animals:* Animals can be natural hosts of certain AAV viruses. Therefore, potential hazards to animals that are exposed to the clinical vector shed by the clinical trial subject cannot be ruled out. Considering that there is no known pathology associated to AAVs and that no hazardous insert is present in the clinical vector, the hazards associated with the release of replication competent AAVs can be regarded as very low.

2.2. Exposure characterisation

Likelihood of adverse effects linked to the recombination/mobilisation

The recombined particles would have the *rep* and *cap* genes but they would still be replication-defective (as the wild-type virus). Therefore, the only mechanism by which there could be mobilisation is that the same cell was infected simultaneously with the clinical vector, a wild-type AAV virus and a helper virus (triple infection). The likelihood of simultaneous triple infection can be considered very low.

Likelihood of adverse effects linked to the long-term persistence of the clinical vector within transduced cells (latent infection)

Possible scenarios:

- (1) Accidental transfer to non-target individuals: A possible scenario of accidental transfer to thirds would be in case of a needle-stick accident during administration or accidental exposure during handling of the product by health care professionals. The investigational medicinal product is administered by trained professionals in a highly controlled

² The risk of insertional mutagenesis for the patient should be balanced against the expected benefits for the patient. The assessment of risks for the patient is done under the clinical trial application and is not specifically considered in the ERA.

environment, which minimizes the probability that an accidental transfers can occur during the administration/handling of the product. Taking this into account, the likelihood for the hazard to occur can be considered very low.

- (2) Transmission via shedding: Close contacts of the clinical trials subject could be contaminated with the AAV clinical vector through *e.g.* the saliva, blood, tears, semen, urine or faeces of the clinical trial subject. Depending on the characteristics of the clinical vector and the route of administration, the likelihood of shedding can be low to moderate. However, their quantitative exposure to the clinical vector would be much lower than the clinical dose. Therefore the overall likelihood for the hazard to occur can be considered low.
- (3) Donation of blood, cells, tissues or organs: The exclusion criteria for donations,³ place limitations on the ability of subjects to donate. If a patient treated with an AAV clinical vector became donor of blood, cells, tissues or organs, the receptor of the donation could be exposed to the AAV clinical vector. However, in such scenario, the quantitative exposure to the clinical vector would be much lower than the clinical dose. Therefore the likelihood for the hazard to occur can be considered very low.

Likelihood of adverse events linked to insertional mutagenesis

A causal link between infection by recombinant AAVs and insertional mutagenesis is a theoretical possibility that has been investigated in preclinical studies but a causal link has not been established to date. Likewise, a causal link between the administration of medicinal products based on AAVs and insertional mutagenesis has not been established in any of the clinical trials conducted with AAV clinical vectors so far.

The probability that insertional mutagenesis could occur in the three scenarios of accidental transfer/unintended exposure to the AAV clinical vector above-referred can be considered negligible.

Likelihood of adverse events linked to transmission to animals

Animals could become exposed to particles of the clinical vector shed by the clinical trial subject. A possible scenario is a child treated with AAV clinical vector that plays with a pet.

³ Eligibility criteria for donors of whole blood and blood components are laid down in Annex III to *Commission Directive 2004/33/EC of 22 March 2004 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components* (OJ L91, 30.3.2004, p. 25), as amended.

Selection criteria for donors of tissues and cells are laid down in Annex I to *Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells* (OJ L38, 9.2.2006, p. 40), as amended.

Organ and donor characterisation criteria for organs are laid down in the Annex to *Directive 2010/45/EU of the European Parliament and of the Council of 7 July 2010 on standards of quality and safety of human organs intended for transplantation* (OJ L207, 6.8.2010, p. 14).

However, the quantitative exposure to the clinical vector would be much lower than the clinical dose. Accordingly, the likelihood of inadvertent transmission of significant amounts of AAV to animals via shedding is considered very low.

2.3. Risk characterisation

Risks associated with recombination/mobilisation

In the unlikely scenario that cells transduced by the clinical vector became simultaneously infected by wildtype AAV virus and a suitable helper virus, the expected outcome is the production of wild-type AAVs and more vector particles, which would not be able to replicate as they would still lack the *rep* and *cap* genes. Therefore, the risks to the environment and human population can be considered negligible.

Risks associated to the long-term persistence of the clinical vector within transduced cells (latent infection):

Non-target individuals could develop a latent infection of AAV clinical vector in these scenarios:

- (1) Accidental transfer to non-target individuals: despite the wide prevalence of AAVs in the population, they have not been associated with any disease in humans. In individuals with preexisting immunity (T cells and Ab) to AAVs, clinical vector molecules would be eliminated by the immune system. Accordingly, no/negligible adverse effects linked to the administration of the clinical vector to non-target individuals are expected as the transgene is not associated with any pathogenic properties.

In the case of immune-compromised individuals, it is noted that many patients that have been administered AAV therapies have been pre-conditioned with steroids for weeks/months with a view to suppress immune response to the AAV clinical vector. In this scenario of induced immunosuppression, safety concerns (for the patient) linked to the administration of the clinical vector have not been identified.

In light of the above and of the very low probability of accidental transfer to non-target individuals, the risk associated to latent infection in case of accidental transfer of the clinical vector to non-target individuals can be considered negligible.

- (2) Transmission via shedding: Given the low infectivity rate of recombinant AAVs, a high titre would be required for efficient transduction of the cells. It is therefore very unlikely that the particles shed would have the capacity to cause significant infections in humans.

Furthermore, AAVs are not known to cause disease in humans. Moreover, as no toxic/harmful properties have been identified related to the expression of the transgene, the risk associated to latent infection in case of exposure to clinical vector particles shed from the trial subject is considered negligible. Even if the close-contacts contaminated with AAV clinical vector shed by the clinical trial subject were immuno-compromised,

the risk is expected to be negligible due to the non-pathogenic nature of AAVs and the limited quantitative exposure.

- (3) Donation of blood, cells, tissues or organs: Given the low infectivity rate of recombinant AAVs, a high titre would be required for efficient transduction of the cells. It is therefore unlikely that the amount of particles that could be transferred in case of a transfusion or a transplant could cause significant infections in recipients of the transfusion/transplant.

Therefore, considering the non-pathogenic nature of AAVs and the limited quantitative exposure, the risk associated with the possibility of latent infection in non-target individuals who receive blood, cells, tissues or organs from a patient treated with an AAV clinical vector, is considered negligible.

Risk associated with insertional mutagenesis:

Insertional mutagenesis in individuals other than the clinical trial subject could arise following exposure to an AAV clinical vector in these three scenarios: accidental transfer, shedding or donation of blood, cells, tissues or organs.

Considering that:

- tumour formation associated with AAV clinical vectors has not been reported in patients treated with AAV clinical vectors (including patients that have been immunosuppressed),
 - the quantitative exposure in the three scenarios above cannot be greater than the clinical dose,
 - clinical vector molecules are expected to be eliminated by the immune system of the concerned individual,
- the risk of insertional mutagenesis to non-target individuals can be considered negligible.

Risk associated with transmission to animals

AAVs are not known to be pathogenic to animals. Given the low infectivity rate of recombinant AAVs, a high titre would be required for efficient transduction of the cells. It is therefore very unlikely that the particles shed would have the capacity to cause significant infections in animals. Moreover, there is no hazardous gene product present in the AAV clinical vector.

The risk associated with the transmission to animals is therefore considered negligible.

2.4. Risk management strategies

The applicant should consider if risk management strategies should be implemented to minimise the likelihood of accidental exposure of health care professionals at the clinical trial site. These should be listed in Section 3.6 of the common application form.

2.5. Determination of the overall risk and conclusions

Provided that the control measures described by the applicant are implemented, the overall risks for human health and the environment can be considered negligible.