ZKBS
Zentrale Kommission
für die Biologische Sicherheit

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General statement of the ZKBS genetic engineering work carried out too frequently with the underlying criteria of comparability:

Gene transfer with mastadenoviruses from primates

1. Description of the adenoviral system

1.1 General introduction

Human adenoviruses are widely distributed. As a rule, children already have antibodies against at least one adenovirus type after the first year of life, and after the age of 15 most individuals have antibodies against several different adenovirus types. Adenovirus type 5 (Ad5) and other types (e. g., 1, 2, or 6) may persist in lymphoid tissues for long periods of time. The occurrence of such infections is the cause of the very high antibody titres to these types present in most individuals. Adenoviruses (AdV) frequently cause infections of the respiratory tract in humans, but can also cause rarer clinical pictures such as keratoconjunctivitis, meningitis or pneumonia. [1, 2] .

Human cells are normally permissive for AdV, their infection proceeds productively. Rodent cells are either non-permissive or semi-permissive for some human AdV. [1–5] . For new-born rodents, certain AdV may have neoplastic transforming potential. This characteristic, in addition to serological criteria, was previously used to classify [1, 2] . More recently, data on phylogeny and genome organization have been increasingly used for assignment to the genus *mastadenovirus* and also for assignment to a species. However, according to the *Human Adenovirus Working Group* (HAdV Working Group), an unambiguous classification is difficult [6]. The *International Committee on Taxonomy of Viruses* (ICTV) recommends a phylogenetic distance of > 5 - 15 % as a prerequisite for distinguishing between two AdV species. In addition, other criteria include GC content, neoplastic transforming potential in rodents, host tropism, cross-neutralization, ability to recombine, number of virus associated (VA) RNA genes, and hemagglutination. According to these criteria, human adenoviruses (HAdV) are classified into seven species (*human mastadenovirus A* to *G*), each belonging to several types. Many of the AdV isolated from non-human primates (Simian mastadenovirus, SAdV) are also classified as HAdV, e. g. AdV from chimpanzees, gorillas and bonobos. 7] . In addition, the ICTV lists nine species in which AdV isolated exclusively from non-human primates are found (*simian mastadenovirus A* to *I*) [8] . However, a clear delineation of the species isolated from humans and from non-human primates is not always accurate, as recombinants between human and simian AdV can also occur. [9] . Accordingly, the present statement describes gene transfer with mastadenoviruses from primates (HAdV and SAdV), which are grouped under AdV in the following text for convenience.

The genome of AdV from primates consists of a double-stranded linear DNA with a length of 32 - 36 kb (Fig. 1a.). At the ends of the DNA are inverse terminal repeats (ITR) with the polymerase binding sites for the start of DNA replication, followed by the DNA packaging signal Ψ. The "early" events of the productive infection cycle begin with the transcription of the E1a gene, whose gene product transactivates the expression of the other early viral genes E1b, E2, E3, and E4 and initiates an expression cascade of the early genes. Early transcription is followed by DNA replication, which initiates the "late" phase of the productive infection cycle. In the "late" phase, the "late" genes, which mainly encode structural proteins of the icosahedral capsid, are expressed and particles are formed. The productive cycle leads to lysis of the infected host cell [1, 2] .

The E1a proteins interact with a variety of cellular proteins and have several functions during adenovirus infection. They activate transcription of the early genes E1b, E2, and E3, lead to neoplastic transformation in rodent cells, and can stimulate quiescent cells of G0 or G1 phase to enter S phase. The genes encoded by E1b inhibit the p53-dependent induction of apoptosis [1, 2] . The E2 region encodes three proteins required for viral DNA replication, the preterminal protein pTP, DNA polymerase (Ad Pol), and an ssDNA-binding protein (DBP) [2] . The gene products encoded by the E3 region are not essential for replication *in vitro,* but are involved in modulating host defence against infection. E3 encodes several proteins that, among other things, block the transport of the major histocompatibility complex to the plasma membrane or inhibit the lysis of adenovirus-infected cells by tumor necrosis factor TNF [1, 2] . The E4 region encodes several spliced mRNA, and the expressed proteins are named after the ORFs of the E4 region (e. g., E4orf1 or E4orf2). They also have various functions such as stimulating protein synthesis by activating the protein kinase mTOR, protecting genome ends by inhibiting the cell's own repair mechanisms for double-strand breaks, or stimulating transcription of the E2 ORFs [2] .

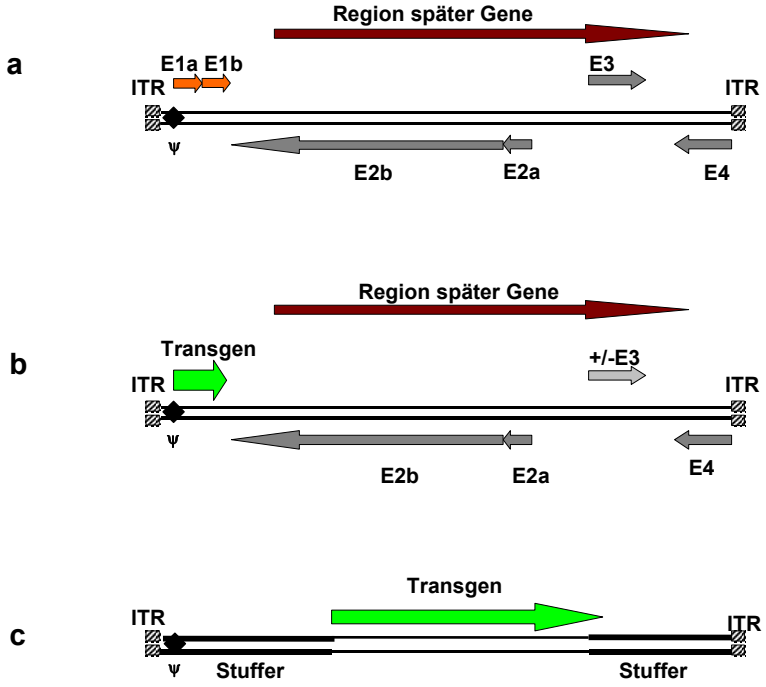

Fig. 1 Genome map of adenoviruses and derived vectors

a. Genome map of adenoviruses. The early transcription units E1a, E1b, E2a, E2b, E3, E4 and the region of the late genes are shown in transcription direction, the inverse terminal repeats (ITR) and the packaging signal ψare marked.
b. Genome map of adenovirus-derived vectors with first generation foreign gene. The E1 region is deleted and replaced by a foreign gene (transgene). The E3 region may additionally be deleted.
c. Genome map of adenovirus-derived gutless vectors with foreign gene. All viral coding nucleic acid segments are deleted (gutless vectors) and replaced by a foreign gene and by functionless filling nucleic acid segments (stuffer).

Figure modified after [10] .

1.2. Gene transfer using AdV vectors

AdV-derived vectors are infectious, replication-defective particles with DNA portions of AdV that can transmit foreign DNA.

The versatile use of AdV vectors is based on their favourable properties. They exhibit high transduction efficiency both *in vitro* and *in vivo*, infect a variety of different cell types, including non-dividing cells, and can be grown in a high-titre manner. AdV vector particles are used both *in vitro* to deliver heterologous genes and in *vivo* as vaccines or in the context of somatic gene therapy [11–15] . The adenovirus capsid can package up to 105% of the length of the wild-type genome; for Ad5, with a genome length of approximately 36 kb, this translates into an uptake capacity of approximately 1.8 - 2.0 kb of foreign DNA [16] . When using deletion mutants, correspondingly larger DNA fragments can be introduced into the AdV genome. AdV vectors whose E1 region is deleted are often used for gene transfer. Additionally, in some of these vectors, the E3 region is deleted to increase uptake capacity ("first generation adenoviral vectors", see Figure 1b). Deletion of the E1 region results in replication-defective adenoviral vectors. The defective viral genome transferred from adenoviral vector particles to non-complementing target cells normally dwells episomally, resulting in only transient expression of the transferred gene (foreign gene). No production of new viral particles occurs.

1.3 Preparation of AdV vector particles

Recombinant adenoviral vector particles can be produced by various methods. The nucleic acid segment to be transferred can be inserted into the AdV genome by direct ligation. As a rule, however, homologous recombination between the nucleic acid segment to be transferred and the viral genome is the basis.

<u>Homologous recombination in a helper cell line</u>

Very often, homologous recombination is induced in a human cell line that is permissive for adenoviral replication. Here, an AdV genome is transferred to human cells together with a recombination plasmid containing the foreign gene flanked by AdV nucleic acid segments. A complete genome, an E1-deleted genome, or an AdV genome containing other deletions of early genes in addition to the E1 deletion can be transferred. The viral DNA replicates through an E1 region, which may be provided separately, and recombination occurs between the recombination plasmid and the viral genome via DNA sequence homologies. Thus, the foreign gene is integrated into that position in the viral genome which is predetermined by the DNA sequences flanking the foreign gene. The recombinant viral genome is packaged by the late viral proteins and delivered as AdV vector particles with foreign gene from the cell line (Fig. 2).

The foreign gene is mostly inserted in place of the E1 region, making the AdV vector particles replication defective. However, viral DNA replication requires the E1 gene products, which

must be provided in *trans*. For this purpose, permissive cell lines are used into which the E1 gene has been previously genomically introduced and which subsequently constitutively express the E1 gene [17–19] . These helper cell lines not only enable the production of E1-deleted adenoviral vector particles, they are also required for the propagation of these particles.

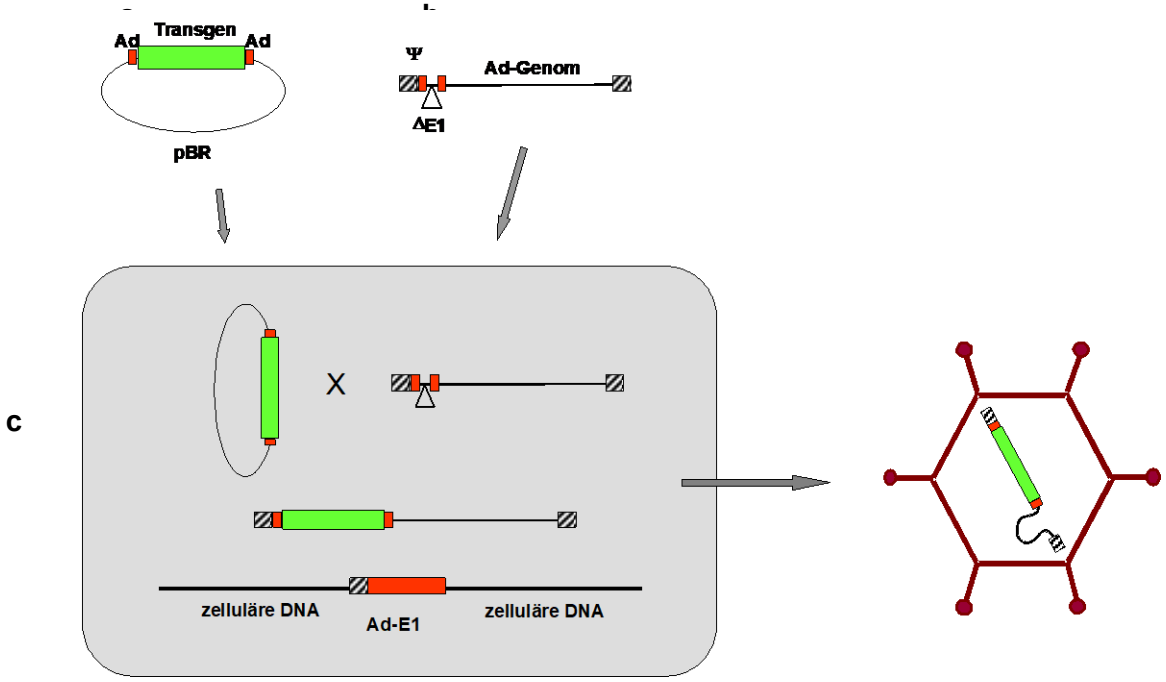

Fig. 2 Production of a replication-defective adenoviral vector particles including a foreign gene in a helper cell line

The preparation of adenoviral vector particles (d) is carried out in several sub-steps:

a. The foreign gene (transgene) to be integrated into the AdV genome (Ad genome) of the viral vector (b) is first introduced into a pBR-derived recombination plasmid (a). There it is flanked by nucleic acid segments (Ad) homologous to the non-coding 5' and 3' regions of the AdV E1 region.
b. In the AdV genome, the coding nucleic acid segments of the early genes E1a and E1b are deleted (ΔE1). Adjacent non-coding 5' and 3' regions are still present. The defective AdV genome can be present either in a vector particle or integrated in a plasmid.
c. The recombination plasmid containing the transgene (a) is introduced into a helper cell line together with the defective AdV genome (b). The helper cell line contains the 5' end of the AdV genome integrated with the E1 region and expresses the E1 gene products, complementing the replication defect of the co-transfected AdV genome. In the course of viral DNA replication, recombination occurs between the recombination plasmid and the defective AdV genome via the DNA sequence homologies of the non-coding regions of E1. This results in a replication-defective AdV genome in which the transgene is now present instead of the deleted E1 region.
d. The late genes still present in the AdV genome are expressed. Their gene products package the AdV genomes with and without foreign genes into replication-defective infectious particles that are delivered by the helper cell line.

Homologous recombination in *Escherichia coli*

In this method, to transfer the foreign gene to the AdV genome, the efficient recombination system of the *E. coli* K12 derivative BJ5183 (*recBC sbcBC*) [20] was used. Two different DNA constructs that are supposed to recombine with each other are transferred together to *E. coli* BJ5183. One of these DNA constructs carries the genome of an AdV to which the foreign gene is to be transferred. The genome is either complete, with a deletion of the E1 region or with deletions of the E1 and E3 regions. The other DNA construct contains the foreign gene. It is flanked by AdV nucleic acid segments originating from the genome region to which the foreign gene is to be transferred. The foreign gene is mostly inserted in place of the E1 region, making the AdV vector particles replication defective. Homologous recombination in *E. coli* BJ5183 results in a plasmid with an AdV genome in which the foreign gene is inserted [21, 22] .

AdV genomes are excised from recombinant plasmids generated in *E. coli* BJ5183 and transfected into a helper cell line that provides the E1 proteins necessary for viral replication. Replication-defective AdV vector particles containing foreign genes are generated and delivered.

Gutless vectors

The replication defect of first generation adenoviral vectors was achieved by deleting the E1a and E1b genes. To increase the uptake capacity, the E3 gene was additionally deleted in some of these vectors. However, they still contain the other early and the late viral genes, which can be expressed in small amounts after infection [23, 24] . In the case of second-generation adenoviral vectors, which also have deletions of the E2 and E4 regions, the late genes are still present. When used clinically, the viral gene products induce an immune response against the transduced cell, resulting in inflammation and a shortened expression period of the foreign gene. Also, the observed minor replication of vector DNA in target cells is due to weak expression of viral genes [25–27] .

In the case of the so-called gutless or high capacity vectors, all viral reading frames have been deleted, so that the immune response against the vector is reduced and the uptake capacity for foreign DNA is increased. [28–32] . In these vectors, only viral nucleic acid segments are still present, which act in *cis and* are essential for DNA replication and packaging of the viral DNA: The ITRs containing the polymerase binding sites for the initiation of DNA replication and the DNA packaging signalΨ . Between the two ITRs, the original region of the adenoviral genes is exchanged for non-coding padding DNA (stuffer). In the resulting vector particles, the foreign gene is partially inserted in place of the stuffer DNA (see Figure 1c and Figure 3b).

The gutless or high capacity vectors rely on a helper virus for their production, which provides the proteins necessary for viral replication and packaging. Such a helper virus is then present, as expected, as a contaminant of the generated vector particle and can be efficiently separated.

One method for producing gutless vectors is the Cre/loxP helper-dependent system derived from bacteriophage P1. This has the advantage that a replication-defective helper virus can be used, which can be separated very efficiently. In addition, the replication defect contributes to the safety of the system [30, 31]

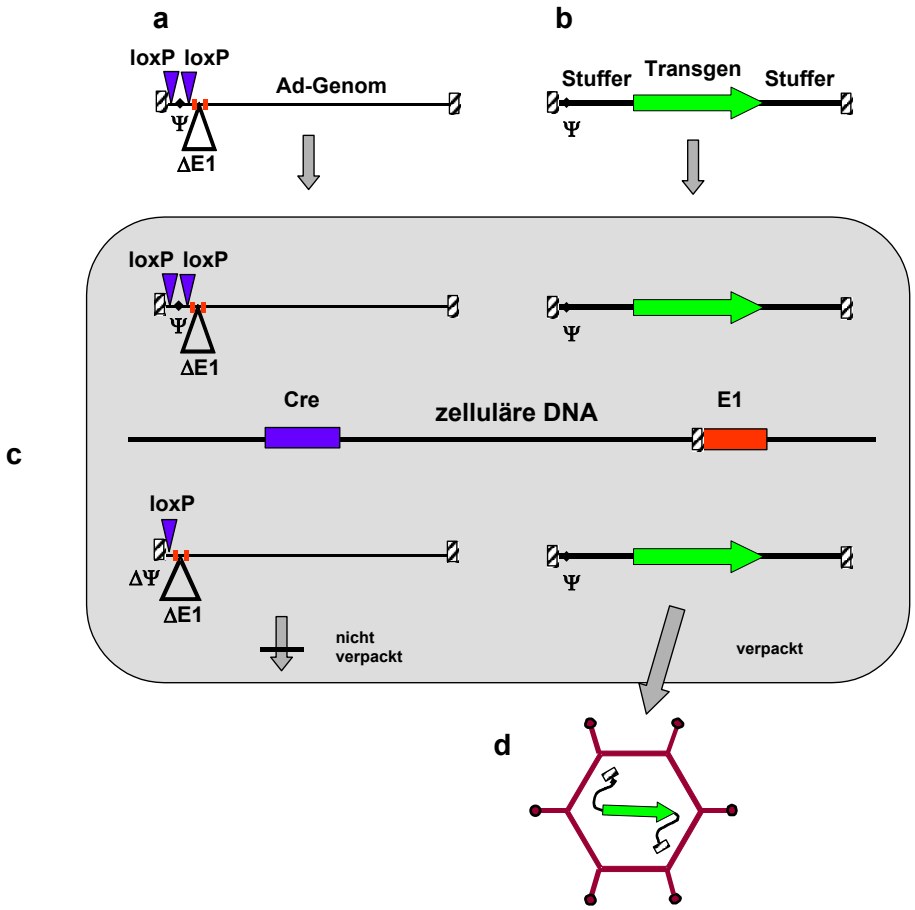

Fig. 3 Generation of gutless vector particles including a foreign gene with the aid of the Cre recombinase system

a. The packaging signal of an AdV helper virus genome (Ad genome) is flanked by two *loxP* nucleic acid segments that are recognized by Cre recombinase. The E1 region and possibly also the E3 region are deleted, all other adenoviral genes are still present.

b. The foreign gene to be transferred (transgene) was introduced into the gutless vector DNA and is present there along with non-coding padding Ψnucleic acid segments (stuffer), the ITRs of the AdV and the packaging signal.

c. The AdV helper genome (a) and the gutless vector DNA (b) are introduced together into the helper cell line 293-Cre. In the helper cell line genome, the genes of Cre recombinase (Cre) and an AdV-E1 (E1) are present in an integrated manner and are constitutively expressed. AdV-E1 complements the E1 deleted in the helper virus genome. Thus, the remaining adenoviral genes of the helper virus genome are expressed. Both the helper virus genome and the *gutless vector DNA* replicate. Cre recombinase excises the packaging signal of the helper virus genomeΨ, rendering helper virus genomes unpackable, whereas gutless molecules with a packaging signal are packaged into virus particles. Ψ

d. The viral gutless vector particles with foreign gene are released from the helper cell line.

The figure was modified according to [30] .

The Cre/loxP helper-dependent system uses three components: an AdV helper virus, a gutless vector DNA and a helper cell line. The AdV helper virus is an E1-deleted AdV in whose genome the packaging signal Ψ is flanked by *loxP* nucleic acid segments. In most cases, a marker gene is present inserted between the *loxP* nucleic acid segments in addition to the packaging signal Ψ. The *loxP* nucleic acid segments are recognition sequences for Cre recombinase, an enzyme of bacteriophage P1. After infection of a helper cell line (293-Cre) to which the *cre* gene has been transferred and which expresses the Cre recombinase, the packaging signal Ψ of the helper virus DNA and the marker gene are excised. If, in addition, the gene products of the E1 gene missing from the helper virus are still present in this helper cell line, all viral genes still present on the helper virus genome are transcribed and the helper virus genome is replicated. If a gutless vector DNA is also transferred into this helper cell line, which contains the ITRs and the packaging signal Ψ of an AdV in addition to the foreign gene and the replenishing non-coding stuffer sequences (see Figures 1 and 3), the gutless vector DNA is also replicated by the viral proteins provided by the helper virus. Since the gutless vector DNA still has the packaging signal Ψ, only this DNA is packaged into the viral particle by the viral capsid proteins provided by the helper virus. In contrast, the helper virus DNA can no longer be packaged because its packaging signal Ψ has been excised by Cre recombinase (see Figure 3). Furthermore, CsCl gradient centrifugation offers the possibility of separating any remaining contaminating particles of the helper virus as far as possible, since they sediment at a higher density than the gutless vector particles.

Safety of helper cell lines

Replication-defective adenoviral vector particles and replication-defective helper viruses lack the E1 region. Both their generation and replication require permissive cells that provide the E1 region genes. A commonly used helper cell line is human cell line 293, which was generated by transfecting human embryonic kidney cells with fragmented Ad5 DNA [17] . In the genome of this cell line, there is an integrated copy of the left Ad5 end, which contains the packaging signal Ψ, the late pIX gene and the E1a and E1b genes in addition to the ITR, which are constitutively expressed [17, 18] . Another helper cell line with a comparable E1 integration pattern is 911 [19] .

The Ad5 nucleic acid segments present integrated in these helper cell lines overlap with nucleic acid segments in Ad5-derived, but also other AdV vectors (see Figure 4) or in the helper virus. This raises the possibility of homologous recombination in the helper cell, whereby the E1 gene is transferred back into the DNA of the vector or helper virus, making the vector or helper virus replication-competent. Such homologous recombinations have been previously shown for 293 cells [33–35] . The presence of replication-competent AdV particles in the vector preparations poses a safety risk for both human applications and laboratory work. The AdV vector particles generated in these helper cells without or with foreign genes should be checked for the presence of replication-competent AdV particles. The use of cell lines such as the PER.C6 line may not result in the generation of replication-competent adenovirus particles. These cells contain the E1 region under the control of a heterologous promoter and a heterologous polyadenylation signal (see Fig. 4). They are used in conjunction with AdV vectors that lack the nucleic acid stretches for E1a and E1b, so there is no homology between the helper cell line and the AdV vector [36, 37] .

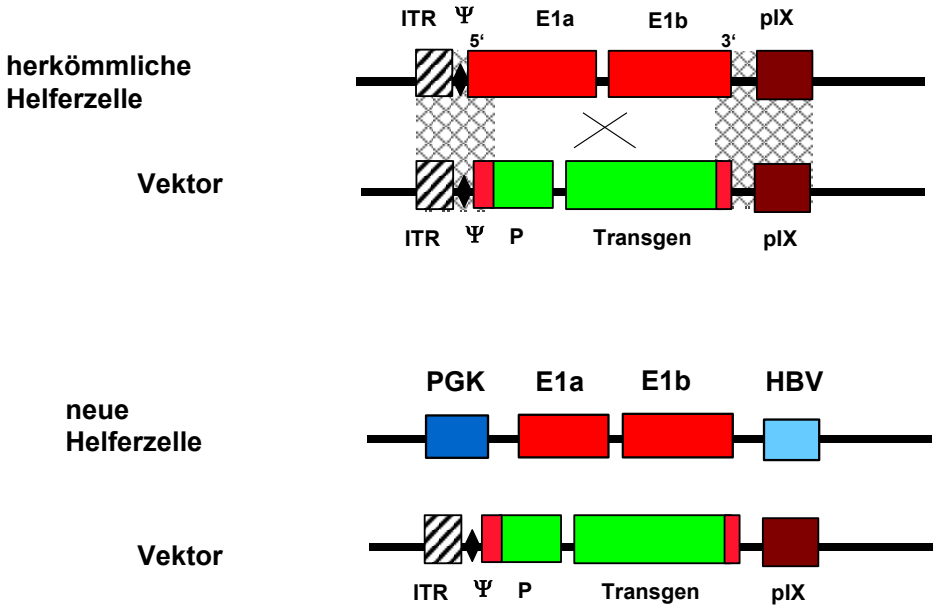

Fig. 4 AdV DNA in helper cell lines and in the vector including a foreign gene
Helper cell lines like 293 mostly contain 5' regions of the Ad5 genome: ITR as well as the genes E1a, E1b and pIX. The non-coding 5' and 3' regions are labelled. The adenovirus vector DNA contains the foreign gene (transgene) with its own promoter (P) instead of the coding region of E1a and E1b, but flanked by E1 nucleic acid segments homologous to the viral DNA of the helper cell line (shaded areas), so that recombination is possible.
The new helper cell lines, such as PER.C6, provide the coding regions of the E1a and E1b genes under heterologous expression control, e. g. by the promoter of the human phosphoglycerate kinase (PGK) gene and the polyadenylation signal of the human hepatitis B virus (HBV) surface protein gene, in *trans*.

The figure was modified according to [36] .

1.4 Gene transfer by AdV vectors in gene transfer complexes

AdV vector particles are also used as a component of gene transfer complexes because of their endosomolytic activity, without themselves containing the gene to be transferred insertively [38] . In addition to AdV particles, such aggregates contain an expression plasmid with the nucleic acid to be transferred, a polycation such as polylysine and transferrin for binding to the cell. AdV mutants that are deleted in the E4 gene are frequently used for this purpose. These E4-defective AdV mutants are complemented and propagated on cells that constitutively express the E4 region.

2. Summary of relevant criteria for the safety classification of genetic engineering work with AdV vectors

In the safety assessment of genetic engineering work with subgenomic and replication-defective nucleic acid segments of AdV in *E. coli* K12 and its derivatives, no risk to human health and the environment is assumed according to the state of the art in science and technology - provided that no further nucleic acid segments with hazard potential are introduced. If, on the other hand, the complete or a replication-competent (e. g. only E3-deleted) genome of an AdV is amplified, even if it is interrupted by nucleic acid segments of the plasmid, the hazard potential of the AdV shall be fully included in the risk assessment.

If the amplified recombinant plasmids are transfected into eukaryotic cells, the decisive factor for the safety assessment is whether AdV vector particles can arise. A low risk potential for humans is assumed, provided that no heterologous nucleic acid segments are present inserted in the AdV genome, which increase the risk potential of AdV. A low risk is assumed when handling AdV vector particles, since they efficiently infect human cells, and since it cannot be excluded that the viral genome - as a rare event - integrates into the host genome. In particular, the possibility of such integration should be considered because the adenovirus vector DNA may be able to replicate marginally due to the presence of remaining viral genes. In gutless vectors, integration is not expected due to the absence of adenoviral genes.

If helper cell lines are used in which the emergence of replication-competent adenovirus particles by recombination cannot be excluded, contamination with such viruses must be assumed.

In the newly developed helper cell lines, in which the helper genes of the E1 region are reduced to the coding nucleic acid segments and in which homologous recombination with the replication-defective AdV genome cannot be assumed, only illegitimate recombination would be conceivable. However, as an extremely rare event, this is not to be taken into account in the risk assessment.

After infection of cell cultures or animals, the replication competence of the AdV vector particles alone is to be evaluated:

- After infection with replication-competent AdV vector particles including foreign gene or after corresponding transfection, new replication-competent recombinant AdV vector particles are generated, which are safety-relevant for the evaluation of the genetic engineering work.
- After abortive infection with non-replication-competent AdV vector particles including foreign gene or after appropriate transfection, no new AdV vector particles are generated unless the infected cells or the infected animal abolish the replication defect of AdV. The viral nucleic acid is not mobilized and transferred to additional cells. When handling these cells or animals, it should be noted that the administered vector particles may be released again, if necessary, because entry into a cell has failed (see point 4.3.).

3. Criteria of comparability of genetic engineering work with AdV vector particles

The following summarises general criteria for comparability in genetic engineering operations involving adenoviral vector particles. Genetic engineering operations with genetically modified organisms (GMOs) that meet the above criteria are comparable with each other and are to be assigned to the safety level that corresponds to the risk group of the GMO.

If genes of prions or toxins are to be transferred by means of adenoviral vectors, a case-by-case evaluation by the ZKBS is required.

If nucleic acid segments with a neoplastic transforming potential are to be transferred, the general comments listed under point 4.6. must be observed.

The following definitions of terms are used:

- **Foreign gene:** prokaryotic, eukaryotic or viral nucleic acid segment that cannot functionally replace the function of the proteins of the adenoviral E1 region and does not code for a prion or toxin.
- **Recombination plasmid**: pBR-derived plasmid with a foreign gene flanked by adenoviral nucleic acid segments homologous to, for example, the non-coding 5' and 3' regions of the AdV E1 region (see p.4 and p.6).

Transfer of plasmids with subgenomic adenoviral nucleic acid segments to *E. coli* K12 derivatives

3.1. *E. coli* K12 derivatives including one or more plasmids containing subgenomic nucleic acid segments of an AdV as well as a foreign gene are **risk group 1** GMOs provided that the vector-receptor system used is a biosafety measure and the adenoviral DNA cannot lead to the formation of replication-competent virus particles in permissive eukaryotic cells.

3.2. *E. coli* K12 derivatives including a plasmid containing the genomic DNA of an AdV and, if applicable, another plasmid containing a foreign gene are **risk group 2** GMOs.

3.3. *E. coli* K12 derivatives including a recombination plasmid are **risk group 1** GMOs**.**

Generation of adenoviral vector particles

3.4. Eukaryotic cells of **risk group 1 to** which subgenomic nucleic acid segments of an AdV and a foreign gene have been transferred by transfection or infection are **risk group 1** GMOs provided that the adenoviral E1 region is not present in the viral DNA or otherwise in the cell. No replication-defective adenoviral vector particles or replication-competent virus particles are formed and delivered.

3.5. eukaryotic cells of **risk group 1 to** which the genomic DNA of an AdV has been transferred by transfection or infection are GMOs of risk **group 2**. replication-competent virus particles of **risk group 2 are** produced and delivered.

3.6. eukaryotic cells of **risk group 1** transfected exclusively with a recombinant plasmid are **risk group 1** GMOs.

3.7. Eukaryotic cells of **risk group 1 to** which coding subgenomic or genomic nucleic acid segments of an AdV and a foreign gene and, if applicable, a recombination plasmid have been transferred by means of transfection or infection are GMOs of **risk group 2,** provided that the proteins of the adenoviral E1 region are expressed in the cells. Replication-defective adenoviral vector particles of **risk group 2** and, if applicable, replication-competent virus particles of **risk group 2 are** formed and delivered.

3.8 Eukaryotic cells of **risk group 1 which** have been infected with a replication-competent helper virus and to which additionally non-coding subgenomic nucleic acid segments of an AdV as well as a foreign gene have been transferred by transfection or infection are GMOs of risk group **2**. Replication-competent virus particles of **risk group 2** and replication-defective adenoviral gutless vector particles, if any, with foreign genes of **risk group 1** are formed and delivered. If the replication-competent virus particles are not separated from the replication-defective adenoviral vector particles, e. g. by density gradient centrifugation, the mixture of particles is to be assigned to **risk group 2.**

3.9. Eukaryotic cells of **risk group 1 that** have been infected with a replication defective helper virus and to which additionally non-coding subgenomic nucleic acid segments of

an AdV and a foreign gene, which is not a nucleic acid with neoplastic transforming potential, have been transferred by transfection or infection are **risk group 1** GMOs, unless it can be assumed that the replication competence of the helper virus has been restored by recombination and the packaging of the replication-defective helper viruses has been prevented due to excision of the packaging signal. If the proteins of the adenoviral E1 region are expressed in the cells, the formation and delivery of replication-defective adenoviral gutless vector particles of **risk group 1 occurs**.

3.10. Eukaryotic cells of **risk group 1** infected with a replication defective helper virus and to which additionally non-coding subgenomic nucleic acid segments of an AdV and a nucleic acid segment with neoplastic transforming potential have been transferred by transfection or infection are GMOs of **risk group 2**. If the proteins of the adenoviral E1 region are expressed in the cells, the formation and release of replication-defective adenoviral gutless vector particles with foreign genes (with neoplastic transforming potential) of **risk group 2 occurs**. Additional safety measures must be taken when handling these GMOs (see 4.6).

Transduction of eukaryotic cells with adenoviral vector particles

3.11. Eukaryotic cells of **risk group 1** to which DNA has been transferred using replication-defective adenoviral gutless vector particles of **risk group 1** or **2 that are not** expected to be contaminated with particles of a helper virus of **risk group 2** are GMOs of risk group **1**, provided that the cells are not infected with a helper virus. No replication-defective adenoviral vector particles or replication-competent virus particles are produced and delivered.

3.12. Eukaryotic cells of **risk group 1 to which** DNA has been transferred using replication-defective adenoviral vector particles of risk **group 2,** which are not expected to be contaminated with replication-competent virus particles of risk group **2**, are GMOs of risk group **1** after completion of transduction, provided that the adenoviral E1 region is not present in the genome of the cells. No replication-defective adenoviral vector particles or replication-competent virus particles are formed and delivered.

3.13. Eukaryotic cells of **risk group 1 to** which DNA has been transferred by means of replication-defective adenoviral vector particles of risk **group 2, in** which contamination with replication-competent virus particles of risk group **2** cannot be excluded, are GMOs of risk group **2** even after completion of transduction. If applicable, replication-competent virus particles of risk group **2 are** formed and delivered.

4. Notes:

4.1. If AdV-susceptible cells are transduced with replication-defective adenoviral vector particles for which, due to the production system, recovery of replication competence by recombination with the genome of a helper virus or the adenoviral E1 region contained in the cellular genome cannot be excluded, the absence of replication-competent virus particles shall be demonstrated before downgrading the work to safety level 1.

4.2. If genes of prions or toxins are to be transferred using adenoviral vectors, a case-by-case evaluation by the ZKBS is required.

4.3. Experimental animals whose somatic cells have been transduced using replication-defective adenoviral vector particles are not GMOs. However, the animals must in principle be regarded as carriers of GMOs. The time during which infectious vector particles remain in the animal is highly dependent on the dose administered and the route of inoculation. In addition, the animals may shed the administered vector particles. However, if it can be

demonstrated with the aid of data or suitable literature for comparable systems that adenoviral vector particles are no longer released from the treated animal after a certain period of time, there is no safety concern if the Land authorities, following a case-by-case assessment, set appropriate time limits on their own responsibility, after which the animals are no longer treated as carriers of GMOs.

4.4. In the case of experimental animals inoculated with replication-defective adenoviral vector particles and for which the data mentioned under 4.3. were not submitted, it can be assumed that a clear depletion of the adenoviral particles has been achieved seven days after inoculation, so that work with the cadavers of these animals does not present a hazard potential exceeding safety level 1. The protection of the legal interests according to § 1 No. 1 of the Genetic Engineering Act is also ensured if the animal cadavers are disposed of in the usual way in laboratory animal husbandry without prior autoclaving. Unless Section 24(1)(3) of the Genetic Engineering Ordinance already applies, the competent licensing and supervisory authority may decide in accordance with Section 2(2) of the Genetic Engineering Ordinance that these animal cadavers are to be disposed of in the normal way in laboratory animal husbandry without autoclaving if it is ensured that the animal cadavers do not enter the food and feed chain.

4.5. Animals to which the transduced cells described in 3.11. and 3.12. have been transferred are not GMOs and are not capable of producing GMOs.

4.6. If nucleic acid segments with a neoplastic transforming potential are to be transferred by means of adenoviral vector particles, reference is made in this context to the following general statements of the ZKBS:

- Statement of the ZKBS: Precautionary measures when handling nucleic acids with neoplastic transforming potential (ref. 6790-10-01, updated December 2016)
- Recommendation of the ZKBS on adenoviral and AAV-derived replication-defective particles that transfer a nucleic acid segment with neoplastic transforming potential (Ref. 6790-10-83, updated April 2020).

Literature

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