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Eidgenössische Fachkommission für biologische Sicherheit
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Risk assessment of activities with oncogenic and cytokine-encoding sequences

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1. Introduction

1.1 Background

In experiments with oncogenic or cytokine-encoding nucleic acid sequences certain processes were triggered in study animals, the extent of which was not expected. For instance, in studies into the development of viral vectored immuno-contraceptive vaccines, infection of mice with the recombinant ectromelia virus (mouse pox virus) containing sequences for mouse-egg-surface protein and interleukin-4 resulted in acute mouse pox accompanied by high mortality¹. Development of malignant tumors was demonstrated in mice following direct application of plasmid DNA containing sequences with oncogenic potential^{2,3}.

Particular caution is required therefore when working with nucleic acid sequences that encode proteins with gene-regulating functions (proteins with oncogenic potential, e.g. transcription factors, GTP-binding proteins, protein kinases, growth factors, etc.) or biologically active gene products (cytokines, growth hormones, toxins). In fact, the French *Commission de Génie Génétique* [Genetic Engineering Commission] lists most cytokines as a "hazard factor"⁵. Taken together, these facts have led to uncertainties regarding classification of work with nucleic acid sequences with oncogenic potential and with cytokines.

These Guidelines issued by the Swiss Expert Committee for Biosafety should provide guidance in classifying work with these sequences and in applying adequate safety measures.

1.2 Definitions

Oncogenes

Oncogenes are gene sequences whose gene products cause cells to be transformed into tumor cells. While it is difficult to provide a more precise definition, the following are considered to be oncogenes¹:

- Viral oncogenes and their cellular homologues (note: viral oncogenes, such as adenovirus E1A and E1B, are not considered oncogenic when expressed in their natural setting),
- DNA sequences that generate tumors in animal experiments,
- DNA sequences that transform mammalian cells *in vitro*,
 - o Immortalization sequences
 - o Growth-regulating sequences
 - o Sequences that result in the loss of contact inhibition or cause cells to become tumorigenic in animal experiments.

¹ Jackson *et al.*, J. Virol. 2001; 75: 1205: Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox.

² Burns *et al.*, Oncogene 1991; 6 (11): 1973-1978: Transformation of mouse skin endothelial cells in vivo by direct application of plasmid DNA encoding the human T24 H-ras oncogene

³ Recommendation of the German Central Commission for Biosafety (ZKBS): Precautions during the handling of nucleic acids with oncogenic potential (in German, allgemeine Stellungnahmen, zellbiologische Themen) <http://www.rki.de/GENTEC/ZKBS/ZKBS.HTM>

⁵ Guideline of the French Genetic Engineering Commission: Definition of hazard classes and risk classes - Methods for determining the hazards and risks associated with the use of GMOs (in French) <http://www.recherche.gouv.fr/commis/genetique/principe/chap2.rtf>

The development of cancer is a multi-stage process that requires the activation of oncogenes and the inactivation of tumor-suppressor genes. Although the introduction of a single gene does not usually result in tumor formation, this has been observed in some cases. Nevertheless, an oncogene that is introduced into a cell in a stable manner brings the cell and its descendants a step closer to the formation of a tumor. Oncogene sequences should therefore be considered as potentially hazardous.

Cytokines

Cytokines are biologically active peptides and proteins. They are used for intercellular communication (signal transmitters). Several hundred known cytokines exist, some of which form complex interdependent networks. Examples include interleukins (including interferons and TNFs), haematopoietins, growth factors, neurotrophins, and chemokines. The pleiotropic actions of immunological cytokines include numerous effects on cells of the immune system and the modulation of inflammatory responses. Cytokines involved in the regulation of the immune system can thus directly modify the pathogenicity of a vector, for which reason various safety-related parameters require special consideration when working with vectors containing such cytokine-encoding sequences.

Post-transcriptional gene silencing by small interfering RNA

Small interfering RNAs (siRNA) are double-stranded RNA molecules 21-23 base pairs long that associate with cellular enzymes to promote specific mRNA degradation, thus mediating RNA interference. These RNA molecules can be introduced into the cell by transfection, or, more conveniently, by transduction of vectors expressing siRNA precursors off an RNA pol III H1 promoter⁶. These vectors have therefore the capacity to knock down the expression of any cognate gene and therefore to include loss-of-function phenotypes, including those for tumor-suppressor genes. Thus, they can act as tumor promoters. The risk associated with the use of such vectors must therefore be evaluated, taking into consideration the function of the targeted gene.

1.3. Legal bases

Activities with expression systems containing oncogenic or cytokine-encoding sequences are considered to be activities involving organisms in contained systems and are therefore regulated by the Swiss Containment Ordinance⁷ (CO). The Swiss Ordinance on Occupational Safety in Biotechnology⁸ (SAMV), which specifies the measures to be implemented for the protection of personnel working with, or exposed to, micro-organisms, also applies.

2. Risk Assessment

An example of a risk assessment procedure is shown in Figure 1. The Containment Ordinance envisages a risk assessment including three steps (Art. 8, Alinea 2):

- a) The assignment of the organism to a group;
- b) Clarification as to whether biological containment systems are used;
- c) The assignment of the activity to a class.

The assignment of the organism to a group forms the basis for the classification of the scheduled activity, and thus for the safety level corresponding to that class. The risk of an

⁶ Brummelkamp *et al.*, Science, 2002; 296:550: A system for stable expression of short interfering RNAs in mammalian cells

⁷ Ordinance of 25 August 1999 on the Contained Use of Organisms, (Containment Ordinance, CO), [SR 814.912](#)

⁸ Ordinance of 25 August 1999 on Occupational Safety in Biotechnology (SAMV), [SR 832.321](#)

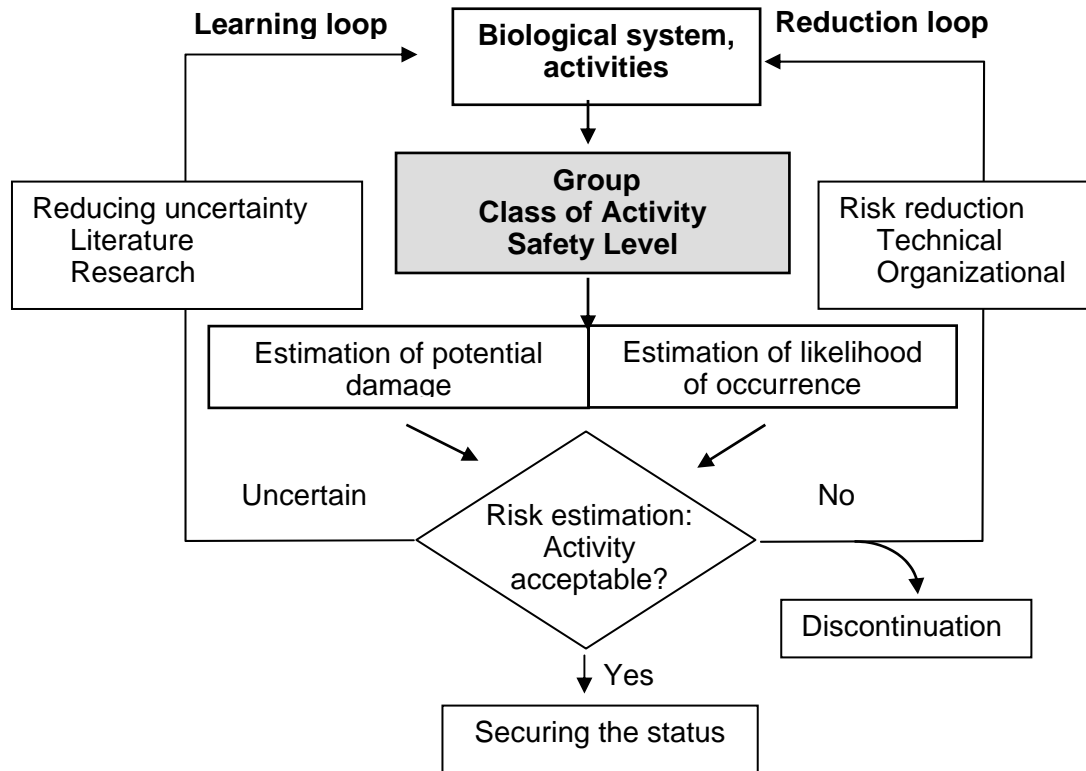


Figure 1: Risk assessment. Assignment of the organism to a group and the activity to a class respectively as required by the CO (shaded) is the basis for a risk estimation of the project.

activity is based on an estimation of the extent of potential damage and the likelihood of its occurrence. For safe applications, i.e. those associated with an acceptable level of risk, personnel should ensure that this status can be maintained. Unacceptable risks can lead to the discontinuation of a project or the reduction of the risk by technical and/or organizational measures (reduction loop). Uncertainties associated with the risk assessment can be reduced through a review of the literature and/or in-house research (learning loop). A definition of the various risk-related terms can be found on the internet-site of the *Office of Hazardous Materials Safety (USA)*⁹

3. General safety considerations for working with oncogenic or cytokine-encoding sequences

3.1 Protective aims

Human health

Negative effects on human health can occur if oncogenic or cytokine-encoding sequences enter, and are expressed in, the human body. Such expression can lead to the formation of tumors in the case of oncogene products or to impairment of the immune response, for example, in the case of cytokines.

⁹ Office of Hazardous Materials Safety (USA), http://hazmat.dot.gov/risk_def.htm

Environment

Negative effects on the environment can arise from the use of expression systems that are also infectious for animals. Such viral vectors, when used in connection with animal experiments, could enter the environment where they possibly could survive and spread.

3.2 Hazard potential

In most cases, the negative effects cannot be accurately predicted. Moreover, they often only manifest themselves during *in vivo* experiments (with animals), which frequently take place only at the end of a series of experiments. Oncogenic or cytokine-encoding sequences used *in vitro* must, in principle, be considered to constitute a hazard potential, although the actual hazard potential should be considered on a case-by-case basis and depends on various experimental factors, particularly on the expression systems used (plasmids, viral vectors). A re-assessment of the hazard potential may be required after completion of the animal experiments (learning loop, see Figure 1).

4. Classification of activities with oncogenic or cytokine-encoding gene sequences

4.1 Use of expression systems

The classification of the expression system forms part of the risk assessment and serves as the basis for evaluating the hazard potential for human health and the environment. Various systems described in a number of documents are being used (incl. adenoviral and retroviral vectors), and these have been assigned to the corresponding classes^{10,11,12,13}. Proceeding from these general classifications, each expression system is analyzed on the basis of the inserts used in the individual case (Figure 2). The insertion of gene sequences that code for cytokines or oncogenes will lead to the same, or higher, classification of the genetically modified organism.

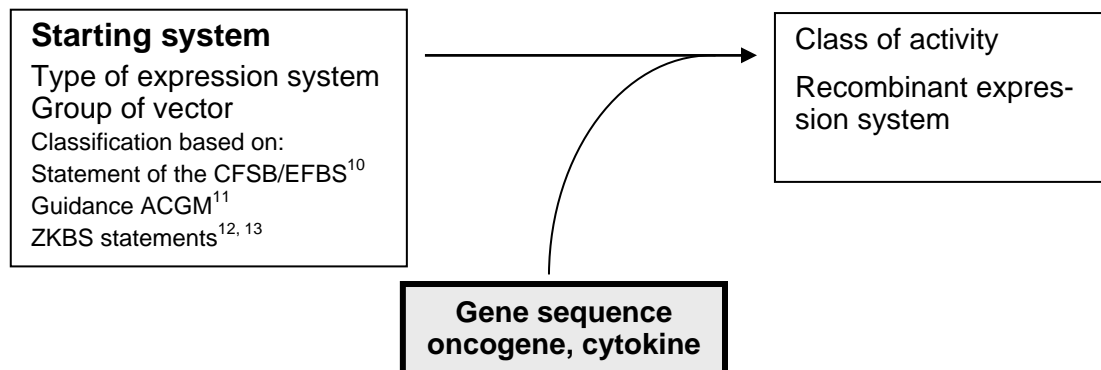


Figure 2: Procedure for determining the class of activity when oncogenic and/or cytokine-encoding gene sequences are used

¹⁰ Statement of the Swiss Expert Committee for Biosafety (CFSB): Classification of work with genetically modified viral vectors (<http://www.umwelt-schweiz.ch/imperia/md/content/efbs/14.pdf>)

¹¹ Statement of the UK Advisory Committee for Genetic Modifications (ACGM): Guidance on commonly used viral vectors (<http://www.hse.gov.uk/hthdir/noframes/acgmcomp/2b3.pdf>)

¹² Statement of the ZKBS: Gene transfer using retroviral vectors (in German) (www.rki.de/GENTEC/ZKBS/VERGLEICHBARKEIT/RETRVEKT.HTM)

¹³ Statement of the ZKBS: Gene transfer using adenovirus type 5 (in German) (www.rki.de/GENTEC/ZKBS/VERGLEICHBARKEIT/ADENO5.HTM)

4.2 Assignment of various expression systems to classes

The classification of activities involving vector systems containing oncogenic or cytokine-encoding gene sequences forms the basis for the safety measures that must be implemented (see Table 1).

The most critical aspects to be taken into consideration for the risk assessment are:

- The tropism of the vector;
- The replication competence of the vector;
- The fact whether a sequence can be expressed in mammalian, in particular in human cells or not.

Depending on the expression system used (vector and recipient organism), the oncogenic or cytokine-encoding gene sequences are placed under prokaryotic or eukaryotic control elements (promoters, terminators, activators). A hazard potential for humans exists, if the vectors used are infectious for human cells, and if the control elements are active in human cell lines and the corresponding genes can be expressed. In this case, negative effects such as tumor formation or impairment of the body's immune defenses could occur.

Table 1: Classification of activities with vectors containing oncogenic and / or cytokine-encoding sequences

	Vector	Activity class
1	Narrow host range plasmids (pBR322 based, such as pBluescript)	Class 1 + organizational measures for the protection of personnel ^{a)}
2	Replication-defective viral vector genome ^{b)} , free or inserted in narrow host range plasmids (pBR322 based, such as pBluescript)	Class 1 + organizational measures for the protection of personnel ^{a)}
3	Replication-competent viral vector genome, free or inserted in narrow host range plasmids (pBR322 based, such as pBluescript)	As for starting organism ^{c)} + organizational measures for the protection of personnel ^{a)}
4	Replication-defective vectors ^{b)} that are infectious for mammalian cells (including human cells and non-human primate cells)	Class 2 + organizational measures for the protection of personnel ^{a)}
5	Replication-competent vectors (excluding group 3 or 4 vectors) that are infectious for mammalian cells (excluding human cells or non-human primate cells, see below)	Class 2 + organizational measures for the protection of personnel ^{a)}
6	Replication-competent vectors that are infectious for human cells or non-human primate cells	Class 3 ^{d)} + organizational measures for the protection of personnel ^{a)}

Notes:

- a) The project leaders or biosafety coordinators can arrange additional personal protective measures such as the use of gloves, safety glasses, and work in a biosafety cabinet (prevention of aerosols).
- b) Examples for replication defective vectors: adenoviral systems, retroviral systems (e.g. MLV-based), lentiviral systems (second or third generation), Semliki Forest virus systems.
- c) *As for starting organism* means: same class as for the vector without oncogenic or cytokine-encoding sequences (see Fig. 2).
- d) The risk assessment should be carried out on a case-by-case basis. A lower classification may be permitted if there is evidence that a particular cytokine or oncogen poses no risk and when the starting vector belongs to risk group 2.

4.3 Notes on the classification according to Table 1

1. *Narrow host range plasmids (pBR322 based, such as pBluescript)*

The use of such plasmids does not significantly affect the hazard potential, independent of whether the insert is regulated by prokaryotic or eukaryotic regulatory control elements. Such expression systems can be assigned to Class 1 (see 5.1, Example 1).

2. *Replication-defective viral vector genome, free or inserted in plasmid*

Since the vector used cannot replicate and is only very weakly infectious for mammalian, including human cells, the hazard potential is not significantly affected. Cloning of such vectors in *E. coli* can be assigned to Class 1 (see 5.2, Example 2).

3. *Replication-competent viral vector genome, free or inserted in plasmid*

Due to the fact that plasmids or free viral genomes are only very weakly infectious for mammalian cells, including human cells, these can be assigned to the same hazard group as the vector without oncogenic and/or cytokine-encoding sequences (*as for initial organism*).

4. *Replication-defective viral vectors that are infectious for mammalian cells (including human cells and non-human primate cells)*

If mammalian cells are permissive for the vectors, there is a possibility that the laboratory personnel may become infected, possibly resulting in the formation of gene products (in this case oncogene products or cytokines) with the corresponding hazard potential for human health. Although such vectors are potentially infectious for human cells, they are unable to replicate and correspondingly, they pose a lower risk. Activities with such vectors can therefore be assigned to Class 2 (see 5.3, 5.4, Examples 3 and 4).

5. *Replication-competent vectors (excluding group 3 and 4 vectors) that are infectious for mammalian cells (excluding human cells and non-human primate cells)*

In these activities, the infectivity and replication competence of the vectors is restricted to animal cells, creating only a low risk for humans. Activities with such vectors can be assigned to Class 2.

6. *Replication-competent vectors that are infectious for human and non-human primate cells*

The hazard potential for humans is at its highest when replication-competent vectors that are also infectious for human cells and non-human primate cells are used for the expression of oncogenic or cytokine-encoding gene sequences. Since replication, and thus a possible increase in the production of gene products, can occur during accidental transmission to laboratory personnel and following release to the environment, activities with vectors of this type are assigned to Class 3.

Replication competence and reversion potential

The replication competence and reversion potential of vectors are important factors in the risk assessment, and thus in the classification of activities and the safety measures to be implemented.

The type of defect (point mutations, deletions, insertions) and the probability of reversion are important in the hazard analysis of replication-defective vectors. The constructs should be reviewed in the individual case and the safety measures adapted accordingly. The recombination of a replication-defective virus - and thus the restoration of replication competence - can occur with the initial virus (e.g. in the personnel) or with the viral sequences of the packaging cell line.

More recent (often commercially available) vectors are usually constructed in such a way as to make both reversion and recombination into a replication-competent vector practically impossible.

5. Examples

5.1 Example 1: Expression of Interleukin-2 or *hras*

Overview of project	Production of interleukin-2 (IL-2) or <i>hras</i> in animal or human cells
Biological system, activities	
Plasmid	pcDNA3.1 (based on the narrow host-range plasmid pBR322, from Invitrogen)
Insert	Human interleukin-2 , under the control of the CMV (cytomegalovirus) immediate-early promoter or other standard regulatory sequences.
Recipient organisms	<i>Escherichia coli</i> BL21 (from Invitrogen, for cloning), CHO (Chinese hamster ovary cells), HEK (human embryonic kidney) (all Group 1).
Resulting recombinant organism	Recombinant <i>E. coli</i> BL21, CHO, HEK cells.
Safety measures	Class 1 + organizational measures for the protection of the personnel: Gloves and safety glasses should be worn and aerosols should be minimized (e.g. by handling the cells in a biological safety cabinet).

5.2 Example 2: Cloning of Interleukin-2 or *hras*

Overview of project	Cloning of interleukin-2 or <i>hras</i> in adenoviral or lentiviral vector
Biological system, activities	
Plasmid	pAdeno-X (description see Example 3); third generation lentiviral vector (e.g. Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D, Naldini L. A third-generation lentivirus vector with a conditional packaging system. <i>J Virol.</i> 1998 Nov; 72(11):8463-71) (all Group 1)
Insert	Human interleukin 2, CMV-promoter, standard regulatory sequences
Recipient organisms	<i>Escherichia coli</i>
Resulting recombinant vector	Recombinant adenoviral and lentiviral vector DNA
Safety measures	Class 1 + organizational measures for the protection of the personnel (as long as not used to transfect human cells or to create virus-like particles): Gloves and safety glasses should be worn and aerosols minimized.

5.3 Example 3: Rational Reprogramming of Mammalian Cells

Project overview	The aim of the project is to obtain cells with biotechnologically and therapeutically usable properties. To this end, replication-defective retroviruses and an adenovirus are used as vectors in order to introduce genes into the cells of interest that influence the cell cycle, differentiation and cell death. The genes are inserted into the cells episomally or are integrated in the chromosomes of the target cells.
Biological system, activities	
Starting plasmids	<p>The vectors to be developed are based on the following plasmids:</p> <p>pMSCVneo (Clonetech): The retroviral sequences are cloned in a pUC-type plasmid and provide the RNA packaging signal and transcription and processing elements (Group 1).</p> <p>pAdeno-X (Clonetech): Approximately 33 kb in length, pAdeno-X viral DNA is derived from an adenovirus type 5 (Ad5) genome that has been altered by deleting extensive portions of the E1 and E3 regions of the Ad5 genome. pAdeno-X is cloned in a pUC-type plasmid (Group 1).</p> <p>pVSV-G (Clonetech): pVSV-G expresses the G glycoprotein of the vesicular stomatitis virus (VSV-G) under the control of the CMV (cytomegalovirus) immediate-early promoter and is cloned in a pUC-type plasmid. VSV-G is used to pseudotype Moloney murine leukemia virus (MoMLV-) based retroviral vectors by mediating viral entry (Group 1).</p>
Inserts	<p>p21, p27: suppressors of cyclin-dependent kinases, which play an important role in the control of the cell cycle. Their inhibition leads to interruption of the cell cycle, stopping the replication and growth of the cells.</p> <p>bcl-xl, bcl-2: survival factors that postpone the death of cells arrested in the cell cycle. Prevention of cell death is a component involved in the survival of tumor cells.</p> <p>CCAAT/enhancer-binding protein α (C/EBP α): a transcription factor that induces and stabilizes the production of cyclin-dependent kinase-suppressors (p21 and p27).</p> <p>Secreted alkaline phosphatase (SEAP): is used as the reporter gene.</p>
Resulting recombinant vector	<p>The resulting retroviral particles are pantropic, i.e. they can infect various cell lines (including cell lines from mammals, insects).</p> <p>The resulting adenoviral particles can infect a series of resting and proliferating cell types of various animal species (including humans, primates, pigs, rodents).</p> <p>In addition, the viral particles contain all, or individual elements, of the following sequences: a suppressor gene, a survival factor, the transcription factor and the reporter gene. The transcription and survival factors are used for inducing and stabilizing the suppressor genes and/or for the survival of the cells arrested in the cell cycle.</p>

Recipient organisms	<p><i>Escherichia coli</i> (laboratory strains, Group 1), cell lines of mice, rats, hamsters and of humans (Group 1 or 2, see statement of the EFBS Guidelines for work with cell cultures (http://www.umwelt-schweiz.ch/imperia/md/content/efbs/54.pdf)).</p> <p>GP-293[pVSV-G] cells: Human packaging cell line for retroviruses, containing the viral <i>gag</i>, <i>pol</i> and <i>env</i> genes (Group 1).</p> <p>HEK 293[pAdeno-X] cells: Human packaging cell line for Adenoviruses, containing the adenovirus E1 gene (Group 1).</p>
Classification of activities	<ul style="list-style-type: none"> - Production (cloning and amplification) of vectors in <i>E. coli</i> (Class 1). - Production of viral particles in GP-293[pVSV-G]-cells and with HEK 293[pAdeno-X]-cells for retroviruses or adenoviruses (Class 2). - Transient infection of various cell lines with the viral particles or stable integration of the retroviral genome in cell lines (Class 2, for as long as viral particles can be detected).
Hazard potential with respect to human health and environment	<p>The starting plasmids are assigned to Class 1. To achieve the project aims, suppressor genes (p21, p27) are expressed in the cell lines. The criteria for determining the hazard potential are:</p> <ul style="list-style-type: none"> - <u>Replication competence of the vector</u> <p>All plasmids used and the expression vectors are replication-defective.</p> <ul style="list-style-type: none"> - <u>Host spectrum of the vectors</u> <p><i>Retroviral vectors:</i> The described expression system generates pantropic vectors, i.e. vectors capable of infecting various hosts (mammals, insects, amphibians) (Class 2).</p> <p><i>Adenoviral vectors:</i> The generated adenoviral particles can infect a series of resting and proliferating cell types of various animal species (incl. humans, primates, pigs, rodents) (Class 2).</p> <ul style="list-style-type: none"> - <u>Functions of the inserts</u> <p>The main function is the interruption of the cell cycles through the introduction of suppressor genes (p21 and p27). Transcription and survival factors are also introduced to help achieve this objective. Viewed as a whole, the effect can be termed anti-tumorigenic, although transcription and survival factors have oncogenic characteristics.</p> <ul style="list-style-type: none"> - <u>Reversion competence</u> <ul style="list-style-type: none"> - The number of reversions to be expected is very small in view of the vectors used (see 4.3) and the small quantities.
Safety measures	<p>Class 2: The planned activities pose a minor threat to human health and the environment. Strict compliance with the regulations for contained systems during the experiments will ensure an adequate level of safety during the application of the biological systems. Skin contact with the organisms or the transfected cells should be avoided by minimizing aerosols (work in a biosafety cabinet), using gloves and or safety glasses.</p>

5.4 Example 4: Analysis of *in vivo* function of Telomerase

Overview of project	Analysis of <i>in vivo</i> function of telomerase by (1) testing the capacity of different mTERT/hTERT chimeras to rescue cells from senescence, (2) determining whether the mouse homologues of other genes encoding for structural or enzymatic telomere components can restore the immortalization function of mTERT, and (3) by using the mTERT-transduced human lung fibroblasts to set up a screen for new genes required for <i>in vivo</i> telomere function.
Biological system, activities	
Plasmid	<p>MoMLV (Moloney murine leukemia virus) retrovirus, e.g. pLAPSN (BD Biosciences): Plasmid containing <i>gag</i>, <i>pol</i> and <i>env</i>-deleted MoMLV genome, replaced by heterologous genes. Multiple recombination events would be required to produce a replication competent virus (Group 1)</p> <p>The MoMLV vectors are replication defective and lack more than two thirds of the viral genome. In the absence of wild-type virus or gene-products that provide the functions of the missing viral genes, the vector is not able to generate a productive infection once introduced into cells or animals. MoMLV does not replicate in humans and the MoMLV virions are exquisitely sensitive to human complement.</p> <p>The MoMLV vector may be substituted by a third generation lenti-viral vector, resulting in the same classification</p>
Inserts	<p>hTERT: human telomerase reverse transcriptase.</p> <p>CD28: chimeric transmembrane protein containing the extracellular domain of mouse IL-4 and intracellular domain of human IL-2 receptor β chain.</p> <p>E6 and E7: HPV (Human Papilloma Virus) viral genes. E6: Transforming protein, binds to the tumor suppressor p53, leading to degradation. E7: Transforming protein, binds to pRB (retinoblastoma tumor suppressor protein).</p> <p>All inserts are apoptosis regulators, in particular preventing cell death. As such, the normal cycling of cells into programmed cell death will be inhibited. This increases the risk of tumor development. It is the breakdown of the regulation of programmed cell death towards prolonged cell survival which is a problem in tumorigenesis.</p>
Resulting recombinant vectors	The constructed viral vectors are able to infect human cells but are replication defective and contain cell regulatory inserts
Recipient organisms	See example 3
Classification of activities	<ul style="list-style-type: none"> - Construction of vectors (cloning and amplification) in <i>E. coli</i> (Class 1) - Production of viral particles with packaging cell lines (Class 2) - Infection of different cell lines (Class 2, once it has been determined that no vector is being shed class 1 is possible)

<p>Hazard potential with respect to human health and the environment</p>	<p>In order to reach the project goals (understand cellular aging) genes active in cell aging and death prevention are transferred into the cells.</p> <p>Important criteria for the hazard assessment are:</p> <ul style="list-style-type: none"> - <u>Replication competence of the vector</u> <p>All plasmids and expression vectors are replication defective. The likelihood of replication is further diminished by observing special experimental precautions (sequential addition of elements involved in replication).</p> <ul style="list-style-type: none"> - <u>Host range</u> <p>The vectors used are able to infect human cells. There is, however, no risk of productive spread of these vectors in humans but there is a small risk of exposure and non-productive gene transfer when handling concentrated stocks.</p> <ul style="list-style-type: none"> - <u>Properties of insert</u> <p>The main function of the inserts is to slow down mechanisms of cell aging and cell death, i.e. the inserts have oncogenic character. Therefore, inadvertent exposure should be prevented.</p> <ul style="list-style-type: none"> - <u>Reversion frequency</u> <p>Multiple deletions and insertion of heterologous genes in place of the replication related sequences lowers the replication frequency significantly. A very low reversion rate can be assumed.</p>
<p>Safety measures</p>	<p>Class 2: The planned activities pose a minor threat to human health and the environment. Strict compliance with the regulations for contained systems during the experiments will ensure an adequate level of safety. Skin contact with the organisms or the transfected cells should be avoided by minimizing aerosols (work in a biosafety cabinet), using gloves and safety glasses.</p>

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