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Swiss Expert Committee for Biosafety SECB

Recommendation of the SECB

on the risk assessment of activities using oncogenic and cytokine-encoding sequences

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1 Introduction

1.1 Background and purpose

Animal experiments with oncogenic or cytokine-encoding nucleic acid sequences were shown to trigger unexpected processes. For instance, studies during which virally vectored immunocontraceptive vaccines were developed resulted in acute mouse pox with high mortality[1], when mice were infected with the recombinant ectromelia virus (mouse pox virus) containing sequences for mouse-egg surface protein and interleukin-4. Development of malignant tumours was demonstrated in mice, following direct application of plasmid DNA containing sequences with oncogenic potential[2, 3, 4, 5].

Particular caution is required therefore when working with sequences that encode for 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 "hazard factors"[6]. This facts make the classification of work with oncogenic or cytokine sequences quite difficult.

This recommendation issued by the Swiss Expert Committee for Biosafety should provide guidance on the classification of work with these sequences and the application of adequate safety measures.

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[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. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC114026/pdf/jv001205.pdf/?tool=pmcentrez

[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] Position statement of the German Central Commission for Biosafety (ZKBS): Precautionary measures in handling nucleic acids with oncogenic potential http://www.bvl.bund.de/EN/06_Genetic_Engineering/ZKBS/01_Allg_Stellungnahmen/10_cell_biology/zkbs_cell_biology_oncogenic_DNA_1991.pdf?__blob=publicationFile

[4] Position statement of the ZKBS: assessment of genetically modified organisms that are to receive nucleic acids coding for gene regulatory functions http://www.bvl.bund.de/EN/06_Genetic_Engineering/ZKBS/01_Allg_Stellungnahmen/10_cell_biology/zkbs_cell_biology_DNA_w_gene_regulatory_functions_1996.pdf?__blob=publicationFile

[5] Position statement of the ZKBS on classifying genetic engineering operations where cytokine or apoptosis-regulating genes are integrated into replication-competent microorganisms http://www.bvl.bund.de/EN/06_Genetic_Engineering/ZKBS/01_Allg_Stellungnahmen/10_cell_biology/zkbs_cell_biology_apoptosis_cytokine_regulating_genes_2002.pdf?__blob=publicationFile

[6] 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) ftp://trf.education.gouv.fr/pub/rechtec/commis/genetique/principe/guide.pdf; List with inserts 2005: http://media.enseignementsup-recherche.gouv.fr/file/CGG/31/0/liste_des_inserts_64310.pdf

1.2 Definitions

Oncogenes

Oncogenes are gene sequences that can cause cells to be transformed into tumour cells. The following examples are considered to be oncogenes:

- 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 tumours in animal experiments,
- DNA sequences that transform mammalian cells *in vitro*,
    - Immortalisation sequences;
    - Growth-regulating sequences;
    - Sequences that result in the loss of contact inhibition or cause cells to become tumourigenic in animal experiments.

The development of cancer is a multi-stage process that requires the activation of oncogenes and the inactivation of tumour-suppressor genes. Although the introduction of a single gene does not usually result in tumour formation, this has been observed in some cases. For example, an oncogene that is stably transduced into a cell might promote the formation of a tumour. Oncogene sequences should therefore be considered as potentially hazardous[7].

Cytokines

Cytokines are biologically active peptides and proteins. They are involved in signal transduction. Several hundred cytokines exist and some of them form complex interdependent networks. Examples are interleukins (including interferons and TNFs), haematopoietins, growth factors, neurotropins, and chemokines. The pleiotropic actions of immunological cytokines also include effects on cells of the immune system and the modulation of inflammatory responses. Such cytokines can thus directly modify the pathogenicity of a vector. Therefore, special consideration is required when working with vectors containing cytokine-encoding sequences.

Post-transcriptional gene silencing by small interfering RNAs

Small interfering RNAs (siRNA) are double-stranded 21-23 base-pair long RNA molecules that interact 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 of an RNA pol III H1 promoter[8]. These vectors have the capacity to knock down the expression of any cognate gene and therefore to include loss-of-function phenotypes, including those for tumour-suppressor genes. Thus, they can act as tumour promoters. The risk associated with the use of such vectors, particularly vector systems that might result in the permanent modification of the host chromatin (for example retroviral and lentiviral vectors) must be carefully evaluated, taking into consideration the function of the targeted gene. According to the SACG Compendium of Guidance[9] one of the features of RNAi knockdown systems is that the targeted gene

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[7] Oncogene data base ZKBS: http://apps2.bvl.bund.de/oncogene/protected/main/oncoGene.do

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

[9] SACGM Compendium of Guidance, Part 2: Risk assessment of genetically modified microorganisms: http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/part2.pdf

is rarely completely silenced. There might be varying degrees of target gene down-regulation and it is therefore important to consider the ramifications of heterogeneous expression of specific genes. siRNA targeted to one defined gene may be able to knock down other genes, due to a certain sequence homology in the mRNA. Therefore human and animal genome databases should be screened for sequence homology.

RNAi experiments may also have deleterious effects upon cellular metabolism due to triggering of cellular antiviral responses and processes. It has been shown that siRNA molecules (even if less than 30nt in length) can trigger the antiviral response to dsRNA. In addition to the degradation of dsRNA molecules, this results in interferon production that leads to inflammation and non-specific inhibition of protein synthesis.

Other siRNA off-target effects are possible[10].

The risk assessment and classification of activities involving regulators such as siRNAs is similar to that which applies to activities involving oncogene and cytokine-encoding sequences and must also be carried out on a case by case study. siRNA will not be further discussed in this recommendation.

1.3. Legal bases and risk assessment

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 Ordinance[11] (CO). The Swiss Ordinance on Occupational Safety in Biotechnology[12] (SAMV), which regulates the measures to be implemented for the protection of personnel working with, or exposed to, micro-organisms, also applies.

According to the CO, a risk assessment has to be performed in order to identify the extent of a potential damage for human beings, animals and the environment, for the biological diversity and the sustainable use thereof must be estimated, as well as the probability of its occurrence. For that purpose, an organism has to be assigned to a group and the corresponding activity to a class. As a consequence, the security measures to be implemented depend from the assessed risks.

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

2.1 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. Expression of such genes can lead to the formation of tumours in the case of oncogene products or to the impairment of the immune response in the case of cytokines.

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[10] Aimee L. Jackson and Peter S. Linsley, Nature Reviews, Drug Discovery, vol. 9, 2010; 57-67: Recognizing and avoiding siRNA off-target effects for target identification and therapeutic application

[11] Ordinance of 25 August 1999 on the Contained Use of Organisms, (Containment Ordinance, CO), SR 814.912, not legally binding English translation http://www.bafu.admin.ch/biotechnologie/01744/index.html?lang=en&download=NHzLpZeg7t,lnp6I0NTU042l2Z6ln1ad1IZn4Z2qZpnO2Yuq2Z6gpJCEdH58fmym162epYbg2c_JjKbNoKSn6A--

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

Environment

Damage to the environment can arise from the use of expression systems that are also infectious for animals or plants. There is a risk that viral vectors used in animal or plant experiments enter the environment where they could survive and spread.

2.2 Hazard potential

In most cases, the negative effects cannot be predicted accurately. Moreover, they often appear only during *in vivo* experiments (with animals), which frequently take place at the end of a series of experiments. Oncogenic or cytokine-encoding sequences used *in vitro* must be considered to constitute a hazard potential and this should be analysed case by case. The risk depends on various experimental factors but particularly on the expression systems used (plasmids or viral vectors). A re-assessment of the hazard potential should be done after completion of the animal experiments.

2.3 Control measures

Given the uncertainty about the hazards of such sequences we recommend to avoid any contact/exposure of the workers to these sequences by:

- Wearing protective gloves;
- Avoiding the use of sharps;
- Minimising aerosol production (safety level 2) and avoiding it (safety level 3).

3 Classification of activities with oncogenic or cytokine-encoding gene sequences

3.1 Expression systems and gene sequences

The classification of the expression system forms part of the risk assessment and is essential for evaluating the hazard potential for human health and the environment. Various systems can be used (incl. adenoviral and retroviral vectors). They are described in a number of documents and have been assigned to the corresponding classes[13,14,15,16,17]. In addition, each gene sequence (insert) must be

[13] Recommendation of the Swiss Expert Committee for Biosafety (SECB): Classification of work with genetically modified viral vectors
http://www.efbs.admin.ch/fileadmin/efbs-_dateien/dokumentation/empfehlungen/09_Viral_Vectors_E.pdf

[14] Statement of the UK Scientific Advisory Committee for Genetic Modifications (SACGM): The SACGM Compendium of Guidance (http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/index.htm)

[15] General position statement of the ZKBS on frequently carried out genetic engineering operations based on the criteria of comparability: Gene transfer using retroviral vectors
http://www.bvl.bund.de/EN/06_Genetic_Engineering/ZKBS/01_Allg_Stellungnahmen/11_comparableness/zkbs_comparableness_retroviral_vectors_2007.pdf?__blob=publicationFile

[16] Statement of the ZKBS: Gene transfer using adenovirus type 5 (in German)
http://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/ZKBS/01_Allgemeine_Stellungnahmen_deutsch/11_Stellungnahmen_Vergleichbarkeit/zkbs_stellungnahmenVergleichbarkeit_Gentransfer_mit_Adenovirus_Typ_5.pdf?__blob=publicationFile

[17] Recommendation of the ZKBS for adenoviral or AAV derived replication defective vectors with cell cycle regulating genes
http://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/ZKBS/01_Allgemeine_Stellungnahmen_deutsch/08_vektoren/zkbs_vektoren_adenovirale_vektoren_mit_onkogenen.pdf?__blob=publicationFile

analysed within the expression system (Figure 1). Taking into account the inserts coding for cytokines or oncogenes will lead either to a similar classification of the recombinant expression system and genetically modified organism (recipient organism), or to a higher one.

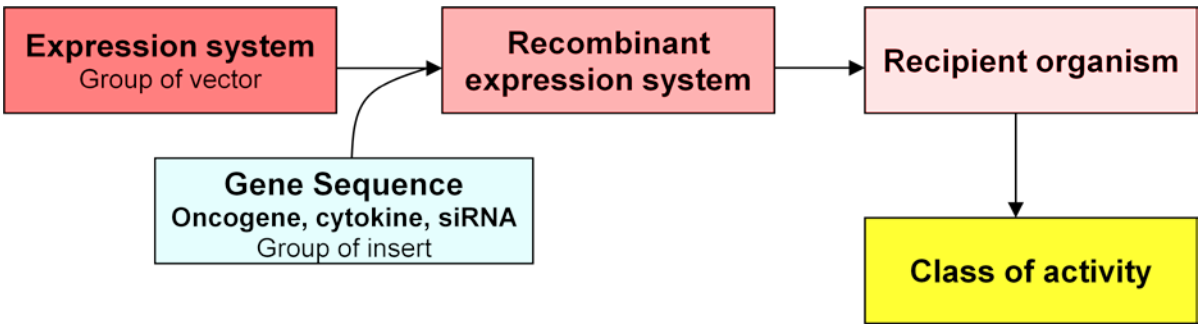

Figure 1: Determining the class of an activity when oncogenic and / or cytokine-encoding gene sequences are used.

3.2 Assignment of expression systems to classes

The risk analysis of activities involving vector systems containing oncogenic or cytokine-encoding gene sequences define the class of activity (see Table 1) and safety measures must be implemented according the ordinance on the contained use of organisms[10].

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 cells, in particular human ones, or not;
- The capacity of the vector system to integrate into the host genome.

Depending on the expression system used (vector and recipient organism), the gene sequences are placed under prokaryotic or eukaryotic control elements (promoters, terminators, activators). If the vectors used are infectious for human cells, if the control elements are active in human cell lines and the corresponding genes can be expressed, there will be a hazard potential for humans. In such cases, negative effects, such as tumour formation, impairment or hyperactivation of the body's immune defences, 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 [a)]
2	Replication-defective viral vector genome[b)], free or inserted in narrow-host-range plasmids (pBR322 based, such as pBluescript)	Class 1[a)]
3	Replication-competent viral vector genome, free or inserted in narrow-host-range plasmids (pBR322 based, such as pBluescript)	Same as starting system [a), c)]
4	Replication-defective vectors[b)] that are infectious for mammalian cells (including human cells and non-human primate cells)	Class 2 [a)]
5	Replication-competent vectors (excluding group 3 vectors) that are infectious for mammalian cells (excluding human cells or non-human primate cells, see below)	Class 2 [a)]
6	Replication-competent vectors that are infectious for human cells or non-human primate cells	Class 3 [a), d)]

Notes:

a) The project leaders or biosafety officers 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, see also the recommendation of the Swiss Expert Committee for Biosafety (SECB): Classification of work with genetically modified viral vectors (http://www.efbs.admin.ch/fileadmin/efbs-dateien/dokumentation/empfehlungen/09_Viral_Vectors_E.pdf)

c) *Same as starting system* means: same class as for the vector without oncogenic or cytokine-encoding sequences (see Fig. 1).

d) The risk assessment should be performed case by case. A lower classification may be permitted if there is evidence that a particular cytokine or oncogene poses a reduced risk and when the starting vector belongs to group 2 or lower.

3.3 Notes on some classifications according to Table 1

Replication-competent viral vector genome, free or inserted in plasmid (No. 3)

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 (same *as starting system*).

Replication-defective viral vectors that are infectious for mammalian cells (including human cells and non-human primate cells) (No. 4)

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 therefore, they pose a lower risk. Activities with such vectors can be assigned to Class 2 (see 5.3, 5.4, Examples 3 and 4).

Replication-competent vectors that are infectious for human and non-human primate cells (No. 6)

The hazard potential for humans is high 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 eventually result in a release to the environment, this type of activities 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 have to be considered 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. Every construct should be analysed separately and the safety measures adapted accordingly. The recombination of a replication-defective virus - restoring the 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 that both reversion and recombination into a replication-competent vector are practically impossible.

4 Examples

4.1 Example 1: Expression of Interleukin-2 or h*ras*

Overview of project	Production of interleukin-2 (IL-2) or h*ras* 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	***Escherichia coli* BL21** (from Invitrogen, for cloning), **CHO** (Chinese hamster ovary cells), **HEK** (human embryonic kidney) (all Group 1).
Resulting recombinant organism	Recombinant *E. coli* BL21, CHO, HEK cells (Group 1).
Safety measures	**Class 1** + organisational measures for the protection of the personnel: Gloves and safety glasses should be worn and aerosols should be minimised (e.g. by handling the cells in a biological safety cabinet).

4.2 Example 2: Cloning of Interleukin-2 or h*ras*

Overview of project	Cloning of interleuking-2 or h*ras* in adenoviral or lentiviral vector
Biological system, activities	
Plasmid	pAdeno-X (description see Example 3); third generation lentiviral vector[18].
Insert	Human interleukin 2, CMV-promoter, standard regulatory sequences.
Recipient organisms	Escherichia coli (Group 1).
Resulting recombinant vector	Recombinant adenoviral and lentiviral vector DNA (Group 1).
Safety measures	**Class 1** + organisational 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 minimised.

[18] 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. J Virol. 1998 Nov; 72(11):8463-71; http://jvi.asm.org/cgi/reprint/72/11/8463.pdf

4.3 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 death. The genes are inserted episomally into the cells or are integrated in the chromosomes of the target cells.
Biological system, activities	
Starting plasmids	The vectors are based on the following plasmids: **pMSCVneo** (Clonetech): The retroviral sequences are cloned in a pUC-type plasmid and provide the RNA packaging signal, transcription and processing elements (Group 1). **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). **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).
Inserts	**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. **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 tumour cells. **CCAAT/enhancer-binding protein α (C/EBP α):** a transcription factor that induces and stabilises the production of cyclin-dependent kinase-suppressors (p21 and p27). Secreted alkaline phosphatase (SEAP): is used as the reporter gene.
Resulting recombinant vector	The resulting retroviral particles are pantropic, i.e. they can infect various cell lines (including cell lines from mammals and insects). The resulting adenoviral particles can infect a series of resting and proliferating cell types of various animal species (including humans, primates, pigs and rodents). In addition, the viral particles contain either 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 stabilising the suppressor genes and/or for the survival of the cells arrested in the cell cycle.

Recipient organisms	***Escherichia coli*** (laboratory strains, Group 1), cell lines of mice, rats, hamsters and of humans (Group 1 or 2)[19]. **GP-293[pVSV-G] cells:** Human packaging cell line for retroviruses, containing the viral *gag, pol* and *env* genes (Group 1). **HEK 293[pAdeno-X] cells:** Human packaging cell line for Adenoviruses, containing the adenovirus E1 gene (Group 1).
Classification of activities	Production (cloning and amplification) of vectors in *E. coli* (**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	The starting plasmids are assigned to Group 1. To achieve the project aims, suppressor genes (p21, p27) are expressed in the cell lines. The criteria for determining the hazard potential are: <u>Replication competence of the vector</u> All plasmids used and the expression vectors are replication-defective. <u>Host spectrum of the vectors</u> *Retroviral vectors*: The described expression system generates pantropic vectors, i.e. vectors capable of infecting various hosts (mammals, insects, amphibians) (Group 2). *Adenoviral vectors*: The generated adenoviral particles can infect a series of resting and proliferating cell types of various animal species (incl. humans, primates, pigs and rodents) (Group 2). <u>Functions of the inserts</u> 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-tumourigenic, although transcription and survival factors have oncogenic characteristics. <u>Reversion competence</u> 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	**Class 2:** The planned activities pose a minor threat to human health and the environment. Skin contact with the organisms or the transfected cells should be avoided by minimising aerosols (work in a biosafety cabinet), using gloves and or safety glasses.

[19] SECB Recommendation on the safe handling of human and animal cells and cell cultures SECB http://www.efbs.admin.ch/fileadmin/efbs-dateien/dokumentation/empfehlungen/10_Zellkulturen_EFBS_E.pdf

4.4 Example 4: Analysis of in vivo function of Telomerase

Overview of project	Analysis of *in vivo* 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 immortalisation function of mTERT, and (3) by using the mTERT-transduced human lung fibroblasts to set up a screen for new genes required for *in vivo* telomere function.
Biological system, activities	
Plasmid	**MoMLV** (Moloney murine leukemia virus) retrovirus, e.g. pLAPSN (BD Biosciences): Plasmid containing *gag*, *pol* and *env*-deleted MoMLV genome, replaced by heterologous genes. Multiple recombination events would be required to produce a replication competent virus (Group 1) 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. The MoMLV vector may be substituted by **a third generation lentiviral vector**, resulting in the same classification
Inserts	**hTERT:** human telomerase reverse transcriptase. **CD28:** chimeric transmembrane protein containing the extracellular domain of mouse IL-4 and intracellular domain of human IL-2 receptor β chain. **E6 and E7:** HPV (Human Papilloma Virus) viral genes. E6: Transforming protein, binds to the tumour suppressor p53, leading to degradation. E7: Transforming protein, binds to pRB (retinoblastoma tumour suppressor protein). All inserts are apoptosis regulators, in particular they prevent cell death. As such, the normal cycling of cells into programmed cell death will be inhibited. This increases the risk of tumour development. It is the breakdown of the regulation of programmed cell death towards prolonged cell survival which is a problem in tumourigenesis.
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	Construction of vectors (cloning and amplification) in *E. coli* (**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)

Hazard potential with respect to human health and the environment	In order to reach the project goals (the understanding cellular aging) genes active in cell aging and death prevention are transferred into the cells. Important criteria for the hazard assessment are: - Replication competence of the vector 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). - Host range The vectors used are able to infect human cells (Group 2). 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. - Properties of insert 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. - Reversion frequency 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.
Safety measures	**Class 2:** The planned activities pose a minor threat to human health and the environment. Skin contact with the organisms or the transfected cells should be avoided by minimising aerosols (work in a biosafety cabinet), using gloves and safety glasses.