European Medicines Agency

London, 8 February 2007
Doc. Ref. EMEA/CHMP/GTWP/125491/2006

COMMITTEE FOR THE MEDICINAL PRODUCT FOR HUMAN USE (CHMP)

Draft

GUIDELINE ON SCIENTIFIC REQUIREMENTS FOR THE ENVIRONMENTAL RISK ASSESSMENT OF GENE THERAPY MEDICINAL PRODUCTS

DRAFT AGREED BY GENE THERAPY WORKING PARTY	**February 2007**
DRAFT AGREED BY BIOLOGICS WORKING PARTY	**January 2007**
DRAFT AGREED BY SAFETY WORKING PARTY	**February 2007**
ADOPTION BY CHMP FOR RELEASE FOR CONSULTATION	**February 2007**
END OF CONSULTATION (DEADLINE FOR COMMENTS)	**August 2007**

Comments should be provided electronically in word format using this template to mailto:GTWPsecretariat@emea.europa.eu

KEYWORDS	environmental risk gmo gene therapy medicinal product deliberate release

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 84 16
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

GUIDELINE ON SCIENTIFIC REQUIREMENTS FOR THE ENVIRONMENTAL RISK ASSESSMENT OF GENE THERAPY MEDICINAL PRODUCTS

EXECUTIVE SUMMARY

This guideline deals with the scientific principles and methodology to be used for the environmental risk assessment (ERA) of GMO-containing gene therapy medicinal products, as required for marketing authorisation (MA) under the centralised procedure. Guidance is given on application of the methodology laid down in the Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms.

1. INTRODUCTION (background)

Directive 2001/83/EC, as amended, and Regulation 726/2004 require that the applicant evaluates the potential risk of the medicinal product to the environment. Therefore an application for marketing authorisation of a medicinal product for human use must be accompanied by an environmental risk assessment (ERA). Procedural guidance for medicinal products containing GMOs appears in the CHMP guideline entitled "Environmental Risk Assessment for medicinal products containing, or consisting of, Genetically Modified Organisms (GMOs)".

During clinical development, national competent authorities are responsible for regulating investigational medicinal products consisting of GMOs. National law and the application of directives for clinical trials differ in the EU member states; however, the information necessary for an ERA should be identical.

2. SCOPE

This guideline provides guidance on the scientific principles and methodology to be used for the ERA of GMO-containing gene therapy medicinal products, as laid down in Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms. The ERA generally needs to consider potential adverse effects for persons (non-patients) directly exposed to the GMO, e.g. staff in the clinic involved in administering the product or in patient care; persons who may be in direct contact with the patient, e.g. family members and the 'general public'; and potential adverse effects

for animals, plants and micro-organisms. The ERA does not concern any effects on the patient being treated.

3. LEGAL BASIS

The legal basis underlying ERAs for medicinal products is outlined in some detail in the CHMP guideline on "Environmental Risk Assessments for Medicinal Products containing or consisting of Genetically Modified Organisms (GMOs)", which is applicable to, *inter alia*, GMO-containing gene therapy medicinal products.

Directive 2001/18/EC requires that an applicant placing any GMO on the market as, or in, a product shall normally submit a Part C notification, including relevant administrative and scientific information, an ERA, a summary, and, if necessary, information on proposed monitoring and risk management strategies, to the designated Competent Authority (CA) of the member state in the territory in which the site intended for placing the GMO(s) on the market for the first time is located. In accordance with a procedure which allows the involvement of the designated GMO CA of each member state and of the European Commission, the notification is examined for compliance with the requirements of the Directive.

European pharmaceutical legislation, in the form of Regulation (EC) 726/2004 and Annex I of Directive 2001/83/EC, require that an applicant for an MA for a biotechnological medicinal product shall submit to the European Medicines Agency a dossier which includes all the necessary administrative, quality, nonclinical and clinical data for the medicinal product. These data are assessed in accordance with the Centralised procedure.

Medicinal products may consist of or contain a GMO. These products constitute a special regulatory case being governed by provisions in both Directive 2001/18/EC and Regulation (EC) 726/2004. These provisions require the environmental impact documentation, including the ERA, to be submitted as part of the medicinal product MA application, and to be assessed as part of the medicinal product Centralised procedure defined in the Regulation.

4. MAIN GUIDELINE TEXT

A marketing authorisation for a medicinal product is given on the basis of a favourable benefit / risk balance. An ERA is based on known facts, including those derived from specific testing of the GMO-containing gene therapy product, and the precautionary principle, which is described in the Communication from the Commission on the Precautionary Principle[Jim Rober1]. During the ERA risks are identified, and if necessary, measures to reduce the risks are defined and a conclusion on the acceptability of the remaining environmental risk is made. The conclusion of the ERA will be taken into account during the final benefit/risk analysis; however this document does not apply to that analysis. For a clinical trial authorisation, the conclusion from a preliminary ERA, adequate for the development stage of the GMO-containing gene therapy product, is taken into account by the relevant EU member state competent authority.

Several general principles apply to an ERA of a GMO-containing gene therapy product.

The GMOs used in gene therapy are either genetically modified cells or they are replication-competent or replication-incompetent viruses that have been modified to deliver genes or genetic material into human tissues or cells. Genetically modified cells that do not harbour replicating recombinant viruses or viruses able to recombine with or mobilize the preventive, in-vivo diagnostic or therapeutic gene(s), may not pose an environmental risk. For genetically modified cells, primarily recombinant viruses administered with or contained within the cells, or potentially resulting from mobilisation or recombination after administration to the subject/patient, need to be considered in the ERA. Therefore, all guidance described below refers to the term GMO which, in this context, refers to a replication-competent or replication-incompetent virus in the gene therapy product, or to recombinant viruses or vectors resulting from recombination with the product following its administration to and release from the patient (shedding).

An ERA should evaluate the characteristics of the GMO and its use in comparison with the corresponding non-GMO or the identical or similar infectious agent present in the environment and its use under corresponding situations (familiarity principle). The objective of an ERA is described in the text of Directive 2001/18/EC.

An ERA should be carried out based on a scientifically sound premise, empirically derived data and/or clinical use. It should be based as much as possible on quantitative and experimental data obtained with the specific gene therapy product. Data in the ERA section of a dossier can be derived from, or supplemented by, data from various sources including the quality, preclinical and clinical sections of the dossier. When quantitative data are lacking it may be necessary, and sufficient, to use qualitative data. Where insufficient data are available to assess the actual risk and where experimental assessment in a suitable model has not led to an acceptable assessment, a realistic but theoretical worst-case scenario can be used. This scenario could be based on the approach developed in the precautionary principle.

New environmental information on the GMO should be evaluated as data become available. When applying for a marketing authorisation or a clinical trial authorisation, the ERA should be up to date and include information from clinical trials, if available. New information becoming available after marketing authorisation should be included in the monitoring plan. Where new data suggest an amendment to the terms of the marketing authorisation is necessary, a variation application should be submitted within the centralised procedure.

Experimental data contributing to the environmental risk assessment of a gene therapy product (shedding and recombinant GMO formation)

Unlike ERA for medicinal products that are chemically derived, there is no action limit for GTMP about which threshold limit may be an environmental risk. There is no threshold for environmental effects, therefore a calculation of environmental risk is based on the probability of transmission of the GTMP from the patient to third party persons, animals, plants or the environment at large. Experimental or clinical observations may contribute to the ERA and it is recommended to incorporate shedding studies in an animal model into the non-clinical medicinal product development program, and also in one or more clinical trials in the clinical development program. For example, as part of the biodistribution studies, urine, faeces or mucosal nasal swabs, could be analysed for the presence of the GMO. The assays used should be state-of-the art and sensitive.

If the presence of a GMO or its genome is detected, shedding and the potential for transmission into the environment may be assumed. Assays used to demonstrate that the GMO is not infectious should have the same or a better sensitivity as those used to detect the presence of the GMO. Only an infectious GMO would be expected to carry a high or medium risk of being transmitted into the environment.

Additional experiments may further characterise the GMO found or suspected to be shed. Such experiments will pave the way for making a decision on whether the risks resulting from its transmission into the environment are low or high. They may be used in any of the steps of the ERA described below.

Methodology for evaluation of the environmental risk (ERA)

The ERA is based on the following key principles, which are further elaborated on in the sections below:

Step 1. Identification of characteristics which may cause adverse effects

Step 2. Evaluation of the potential consequences of each adverse effect, if it occurs

Step 3. Evaluation of the likelihood of the occurrence of each identified potential adverse effect

Step 4. Estimation of the risk posed by each identified characteristic of the GMO(s)

Step 5. Application of management strategies for risks from the deliberate release or marketing of GMO(s)

Step 6. Determination of the overall risk of the GMO(s)

***Step 1*. Identification of characteristics which may cause adverse effects: Any characteristics of the GMO linked to the genetic modification, which may result in adverse effects on human health or the environment shall be identified. A comparison of the characteristics of the GMO with those of the non-modified organism, under corresponding conditions of the release or use, will assist in identifying any particular potential adverse effects arising from the genetic modification. It is important not to discount any potential adverse effect on the basis that it is unlikely to occur.**

Adoption of the precautionary principle and effect on Environmental Risk Assessment

To identify and evaluate possible harmful effects that arise from the use of a GMO, a worst case scenario can be defined. It is important to realise that in a worst case scenario a number of assumptions, for example, assumptions on exposure, are maximised. However, a worst case scenario is useful as it yields a maximum appraisal of the potential hazards. The actual situation, based on the information provided by the applicant, is taken into account.

As an example, a worst case scenario is described for a replication-deficient adenoviral vector with an inserted HSV-tk gene. In the absence of scientific data on the replication-deficiency of the vector in question, a replication-competent adenoviral vector is assumed. This vector contains a functional HSV-tk gene and is able to spread in the environment. It can effectively infect immune compromised individuals. In this scenario it is not expected that thymidine kinase will affect the biology of the vector regarding, for example, replication, systemic distribution, shedding, tissue tropism and host range. Therefore, in the absence of ganciclovir, undesirable effects of the GMO in the environment will be similar to harm caused by a wild type adenovirus, which is present abundantly in the population. It is concluded that in the absence of ganciclovir any harm resulting from an infection with the HSV-tk containing adenoviral vector can be considered small. An infection with this vector in the presence of ganciclovir also needs to be evaluated. The hazard identification must discriminate between two scenarios which differ in the timing of gancilcovir administration.
If ganciclovir is administrated at the beginning of an infection the expression of thymidine kinase will cause apoptosis. Apoptosis will probably kill the thymidine kinase containing cells before new viral particles can be formed. Here, ganciclovir functions as an antiviral drug. Propagation of the vector is cut off early and any harm would be negligible.
Harm caused by induction of apoptosis could however increase with the scale of the viral infection. If for example ganciclovir is administered at the peak of a systemic infection with the vector in question, large amounts of cells and tissues may express thymidine kinase and be killed by ganciclovir. A serious threat for the health status of the infected individual could result. This threat should, however, be offset against the tendency of adenoviruses to kill any infected cell, even without ganciclovir. It is concluded that ganciclovir treatment of systemically infected individuals might cause a harmful effect.

In the above example, the use of a worst case scenario might be sufficient to reach conclusions on the environmental risk. In other cases, a worst case scenario might lead to the conclusion that there is a serious risk to the environment. In that case more information and particularly experimental data should be provided. This information should elaborate on the aspect of spreading of the GMO into the environment as well as on its effect.

The ERA needs to consider the characteristics of the final GMO, taking into consideration the pathogenicity of the unmodified replicating wild type virus (from which the replication-competent GMO or the replication-incompetent GMO was derived) and the nature of the inserted genetic material, which will often be of human origin. Effects on people or the environment posed by GMO-containing medicinal products can be direct or indirect and immediate or delayed. Adverse effects may

be related to inserted genes and their products, but also to an unforeseen change of the host range or tissue tropism of the GMO. All these effects have to be taken into account, either by making theoretical assumptions based on known science or by experimentally assessing pre-requisites or consequences of such effects.

Direct and indirect effects

An example of a direct effect could be accidental exposure to a GMO which is modified to deliver a human growth factor or a bacterial toxin: expression of these genes in non-target tissues could have potentially harmful consequences. However, direct effects may also be delayed in onset, making them more difficult to be linked to the GMO. For example, an immune response against a GMO in a subject other than the treated patient is a direct consequence of shedding. However, such seroconversion may take time to be detectable. Oncogenesis could theoretically be a direct effect of exposure to certain types of GMO but might be hard to link to the GMO if the onset of clinical symptoms occurs at a later date.

An example of an indirect effect is a GMO which compromises prophylactic, therapeutic or diagnostic procedures, through dissemination of antibiotic resistance. Another example could be a new infectious agent arising from recombination or complementation events after release of the GMO.

Hazards associated with the parental / recipient wildtype micro-organism or virus

The risks to the environment from a GMO will depend on the nature of the unmodified wild type virus and the effects of any genetic modification of that virus. For example, when considering a herpes simplex virus based gene therapy product, the characteristics of the GMO will reflect the biology of herpes viruses, and the nature of the genetic material that has been inserted into it. The ERA should therefore consider characteristics including the infectivity, virulence, host entry/infection mechanisms, cell receptors, replication cycle *in vitro*, replication cycle *in vivo*, occurrence of latency, disease or other effects mediated in humans, in animals, in plants, respectively, and mechanisms of transmission.

Other characteristics, such as the stability of the unmodified wildtype virus could be important, and may impact on the ERA. For example, adenoviruses are non-enveloped DNA viruses, are relatively stable, resistant to dehydration and can persist in aerosols and water; whereas herpes simplex virus is an enveloped DNA virus which is highly susceptible to dehydration, and is rapidly inactivated outside of the host. More stable viruses may be more difficult to inactivate using disinfectants and would persist longer in unfavourable environments outside the body, e.g., sewage or non-target hosts. Experimental assessment of the half-life of the GMO in the foreseen environment may therefore be suitable for addressing the persistance of the GMO.

Similarly the host range and tissue tropism may impact on the ERA. For example, some herpes viruses have a narrow host range, whilst several paramyxoviruses have a wide host range. Furthermore, the organism may have been genetically modified to change its host range or tissue tropism, introducing the possibility that new species could be infected. Such engineering may be permanent, e.g., when gene sequences are altered, or temporary, e.g. by pseudotyping. Only permanent effects should be considered as part of the ERA.

An estimation should be made as to the degree of pathogenicity of the host strain and the seriousness of the consequences should exposure occur. Most vectors used in gene therapy are likely to have been modified to reduce pathogenicity, producing an attenuated virus or a replication-incompetent viral vector. For example many adenoviruses have been modified by deletions in the E1 gene, rendering the virus either unable to replicate (replication-incompetent), or to replicate only in certain cells or under certain defined conditions (conditionally replication-competent).

The nature and stability of such attenuating modifications are an important factor to consider in the ERA, as they may significantly reduce or alter the pathogenicity of the gene therapy vector. Usually, the origin and nature of attenuating modifications will be well understood and will form an important part of the risk assessment. In some instances, however the nature of the attenuation may not be well

understood but the wildtype or parent GMO may have a history of safe use. For example, many vaccinia virus strains have been modified by passage, and have not been fully characterised, but have been used extensively in human vaccines.

Safety strategies for retroviral vectors

Transmission of oncogenic risk to third party humans could be a serious harmful effect. Several retroviruses are known to be oncogenic and cause malignant disease, either by insertional mutagenesis of the host chromosomes or as a result of having acquired host oncogenes. Oncogenic retroviral vectors might be derived from replication competent[Jim Rober2] oncogenic retroviruses from the ALV, MoMLV and FeLV subgroups, which are able efficiently to infect actively dividing cells. "First Generation" retroviral vectors are comprised of a packaging system that is essentially a retroviral cDNA itself, encoding viral gag, pol and env genes but with its packaging sequence deleted. This construct is either cotransfected with the transfer vector, or is stably incorporated into the host-cell chromosomes generating a helper cell line. Such systems are inherently the most hazardous since a single recombination event is required to generate RCV. The 3' LTR is deleted in "Second Generation" packaging systems, improving biosafety by reducing the possibility that the packaging construct will be mobilised as well as reducing the likelihood of RCV generation, as two recombination events are required. With "Third Generation" systems, the 5' LTR is also deleted and the packaging sequences are divided between two constructs, with gag/pol encoded by one construct and env by the second. This significantly reduces the likelihood of RCV generation, by increasing the number of recombination events that are required to reconstitute a competent viral genome. Two-component packaging systems of this type should be used wherever possible. Additional biosafety can also be achieved by using self-inactivating (SIN) transfer vectors.

When using replication-incompetent viral vectors or conditionally replicating viruses, the ERA should consider whether it is possible that replicative functions of the virus are restored. For example, recombination events might lead to the formation of a replication-competent virus. Alternatively, complementation by wildtype viruses might allow the GMO to replicate.

Site of insertion of the transgene

For many gene therapy vectors it is common practice to insert the transgene into the site of the disabling mutation. For example, in adenoviruses the gene can be cloned into the deleted E1 region. This adds a level of safety to the vector, because if the virus is restored to replication competence through recombination, the inserted gene would be lost, and the worst-case would be generation of a wild-type adenovirus. For larger viruses, such as herpes viruses, there are usually a number of gene deletions, and insertion of the transgene could be into a number of sites. For such vectors, the ERA should include consideration of the transgene insertion site.

Hazards arising directly from the inserted gene/element

Inserted preventive, *in vivo* diagnostic or therapeutic genes may encode products with defined biological properties which could be harmful if expressed in a different host, or at levels higher than normal, or under conditions in which they would not normally be expressed. For example, many eukaryotic genes involved in cellular signalling, interaction with the environment, cell cycle control, differentiation or apoptosis may be regarded as potentially oncogenic. Also, residual antibiotic resistance genes may interfere with use of antibiotics in the clinic and should be considered in the ERA.

When and how the gene is expressed will affect whether there is any risk to the environment. Stringent control of expression could reduce the environmental risk. Therefore transcriptional control systems need to be described and considered when looking at potential harmful effects of exposure. Expression of potentially harmful genes would not be expected in prokaryotic systems if they were under the control of eukaryotic promoters and vice-versa. Furthermore, inserted sequences can influence the genomic stability of the GMO. When inserted sequences enlarge a viral genome to a size where it is

not packaged efficiently, the genome can be prone to rearrangements. This may lead to unexpected changes in behaviour of the GMO and consequently to unexpected effects on the environment.

Hazards arising from the final GMO: alteration of existing pathogenic traits

Factors which affect pathogenicity or virulence of the host organism. A GMO may contain exogenous genes compared to the parental/wild type organism. Alternatively, there can be deletions to the host genome, affecting the characteristics of the GMO. Many modifications will not involve genes with inherently harmful products but adverse effects may nevertheless arise as the result of exacerbation or alteration of existing pathogenic traits. The following potential mechanisms should be considered, although the list is not exhaustive and all modifications should be carefully assessed in the light of known scientific knowledge.

The modification alters pathogenicity. An inserted gene could encode a pathogenicity or virulence determinant, alternatively the deletion of viral genes that decrease virulence might increase the pathogenicity of the GMO compared to the wild type micro-organism. Where the genetic modifications are expected to be attenuating, the genetic stability of the GMO needs to be considered, as genetic instability could cause the reversion of attenuated viruses to more virulent variants. An insertion or deletion might alter the ability to form latency or reactivation of the GMO. A special case is replication competence. If viral genes essential for replication have been deleted, the ERA should consider the possibility of complementation of missing genes in the patient by a pre-existing infection with wild type virus, possibly leading to enhanced viral shedding and environmental exposure. Also the possibility of recombination events with wild type viruses leading to replicating GMOs should be assessed.

The modification alters susceptibility to the immune system. The ability to evade the immune system is an important determinant of pathogenesis for many micro-organisms. Immune evasion determinants are frequently dispensable for growth *in vitro* and their deletion can be viewed as innocuous or attenuating. In the event that non-patient persons are exposed to the GMO however, it should be considered that whilst the loss of immune evasion function (for example, deletion of E3 from adenoviruses or the IL-18 binding protein from poxviruses) might result in more effective clearing of the GMO during an infection, acute responses such as inflammation may be a feature, and thus the pathogenesis of the GMO could be increased. However, its potential to spread into the environment would consequently decrease. Similarly, insertion of genes encoding immuno-modulatory functions that are not native to the parent micro-organism might affect pathogenesis. For example, poxviruses modified to express interleukin 4 were more pathogenic in an animal experiment than the wild type virus, as this modification led to inhibition of the appropriate immune response for the effective clearance of viral infection. The possible effects of a GMO with impaired immune evasion systems in individuals who may be immunosupressed should also be considered.

The modification alters the tropism of the final GMO with respect to the parental micro organism. There are many factors that might change the natural tropism of a micro-organism. Modification or substitution of viral cellular entry determinants can give rise to viruses with altered cellular tropism. Some viruses (for example vaccinia virus) have a number of host range-determining genes that bestow the ability to replicate within certain cell types. Modifications of viral entry determinants (for example viral surface glycoproteins) might permit the entry of the virus into normally refractory cell types and expression of the inserted sequences might occur, even if replication is impossible. Tropism and host range may also be altered through pseudotyping. Pathogenic bacteria may also have determinants that affect host range or the ability to colonise certain sites. The risk assessment should consider possible effects on tissues not normally infected or colonised by the parent micro-organism and whether the normal route of transmission of the parent micro-organism has been altered. The use of replication-competent viruses with an extended/altered tropism would require greater management control to minimise or prevent wider environmental exposure, for example in hospital staff or family members.

The modification alters the susceptibility of the micro organism to prophylaxis and therapy. In the event of exposure to humans, the availability of effective prophylaxis may be an important containment and control measure. It should be carefully considered, therefore, as to whether the modification will result in reduced susceptibility of the GMO to the prophylactic treatment that is effective against the parent. For example, this could be additional antibiotic resistance bestowed upon bacteria during the modification process or the conferring of drug resistance to a virus (e.g., deletion of poxvirus or herpes simplex virus thymidine kinase functions results in resistance to nucleoside analogue-based antivirals). Furthermore, modifications might result in a GMO that has an altered immungenicity profile. As such workers that are normally immune to the parent micro organism, might therefore be susceptible to infection by the GMO. Moreover, in such cases, a vaccine that protects against infection by the parent micro-organism might not be effective against the GMO.

The modification alters the physical stability with respect to the parental micro organism. This could cause the GMO to be more or less resistant to inactivation procedures.

Transfer of harmful gene sequences from the GMO to be released to humans, plants and animals in the environment

The transfer of genetic material of the GMO released to other humans, plants, animals or micro organisms might be influenced by i) the conditions, e.g. scale and dose, of the release, ii) the availability of susceptible hosts for the gene therapy product, iii) ability of the GMO to replicate and iv) the potential for recombination of the genetic material in the GMO.

During the hazard identification process, it is important to consider the potentially harmful consequences of sequences inserted into a GMO being transferred to other organisms, or that the GMO itself may acquire sequences that might increase its pathogenicity. There are many mechanisms by which sequences may be transferred between organisms. Factors that affect the frequency of gene transfer leading to a harmful consequence are complex and require careful consideration in the risk assessment.

If the sequence is plasmid-borne then it should be considered if the plasmid backbone can be mobilised. Additionally, any selection pressure in the local or wider environment that might contribute to persistence of the transferred gene is of importance. The transferred gene, for example drug-resistance markers, may already be present in nature, diminishing the impact of transfer.

The GMO itself may acquire sequences from the environment. Sufficient consideration should also be given to the possibility that an attenuated or disabled GMO could revert to wild-type status or become competent and able to survive, spread and infect others.

The stability of the genetic modification should also be considered, particularly where there is the possibility that a GMO attenuated or disabled for growth might revert to a wild type or pathogenic phenotype and become an environmental hazard. The genetic stability of the modification might therefore be linked to phenotypic stability, especially where the modification restricts the GMOs ability to survive and to spread.

The loss of an inserted gene from a GMO is unlikely to constitute a hazard per se; however, inherent genetic instability leading to incorporation of genes elsewhere in the genome of the same GMO could be hazardous. Again, it is important to consider that a GMO with a restricted capacity to survive will be under stress in the environment, and there will be a strong selection pressure for the reversion of attenuating and disabling genetic lesions. The possibility that a GMO will be genetically unstable outside of the environment in which it was intended to exist should be taken into account and consideration given to any detrimental effects this might cause.

Recombination between related viruses. Whilst the phenotype of the genetically modified virus is the primary consideration, some thought must also be given to the possibility that harmful sequences may be transferred as the result of a recombination event. Scenarios that need to be considered include the possibility that a disabled vector might recombine with the wild-type virus or with viral

sequences present in the infected cell and revert to a replication-competent derivative of the GMO. One way in which this might arise is as the result of an accidental cross. In many viruses, such as adenovirus, it is reasonable to assume that the repair of the disabling mutation would result in the loss of genetic inserts that are positioned at the site of the disabling mutation. Inserted sequences should be so positioned wherever possible. The decision to insert genes at another site should be fully justified in the risk assessment. However, this may not be the case in larger viruses such as herpes or poxviruses.

Recombination between related viruses

Recombination could for example take place between an adenoviral vector and a wild type adenovirus or viral sequences present in a cell; for example it has been shown that many healthy adults have adenoviral sequences present in their respiratory epithelium. It is common practice to locate an insert in place of the E1 cassette. Thus, any homologous recombination that restores E1 sequences to the vector will also delete the insert and vice-versa. Inserts cloned into other areas of the viral genome could be maintained in the event that E1 sequences are restored, resulting in a replication competent adenoviral vector. In addition other viruses and viral sequences may be able to complement disabled adenoviral vectors. For example, human papillomavirus (HPV) and Epstein-Barr virus proteins have been shown to complement E1A mutant adenoviruses in trans. Therefore in the environmental risk assessment the presence of other viral sequences should be considered.

Step 2. **Evaluation of the potential consequences of each adverse effect, if it occurs: The magnitude of the consequences of each potential adverse effect should be evaluated. This evaluation should assume that such an adverse effect will occur. The magnitude of the consequences is likely to be influenced by the environment into which the GMO(s) is (are) intended to be released and the manner of the release.**

The severity of the consequences of each identified adverse effect needs to be qualified, e.g., in terms of magnitude ranging from high, moderate, low to negligible. These consequences are influenced by the genetic constitution of the GMO, the exposed environment, the health status of those likely to be exposed, the method of administration and the frequency of use of the GMO. Some considerations are:

- The spread of the GMO in the environment or its host species. This might be affected through i) a changed biological fitness (e.g. increased competitiveness), ii) ways of dispersal of viable material (e.g. transport, way of administration, shedding), iii) environmental, e.g., climatological conditions (temperature sensitivity).

- Interactions with other organisms. Examples include the exposure of personnel that are involved in the study and in the treatment of the patient, or the interaction with other micro-organisms that are present in the patient.

- Effects on population dynamics or genetic diversity in the receiving environment, e.g., if a GMO has an increased growth rate compared to wild-type organisms.

Step 3. **Evaluation of the likelihood of the occurrence of each identified potential adverse effect: A major factor in evaluating the likelihood or probability of adverse effects occurring is the characteristics of the environment into which the GMO(s) is intended to be released, and the manner of the release.**

The risk assessment process thus far has involved identifying those features of the GMO that have the potential to cause harm and the mechanisms by which these hazards could be realised. Next, the likelihood that the identified hazards will be manifested should be evaluated. Theoretical scenarios can be drawn up which suggest that a GMO may be hazardous to people, but the chances of these scenarios being realised are often small. The ERA should focus on realistic scenarios.

Factors that come into play are (i) judgement on the overall fitness of the GMO and (ii) the probability that rare events may occur (e.g. the likelihood of gene transfer). Issues relating to the likelihood of harm arising will be very difficult to handle in situations where there is no firm data on which to make a judgement; however, many wild-type viruses have co-existed for thousands of years without jumping species. Nevertheless, caution must be applied when seeking to discount any potentially harmful properties of the GMO on the basis that they are unlikely to be manifested. In general, the credence given to information used in these considerations should reflect the quality of the supporting data. In other words, where there are insufficient data on the GMO at hand, it would be prudent to perform experiments. If the ERA is still incomplete, theoretically assume the worst case scenario in the ERA and act accordingly.

Production strategies

Careful modification of the sequence of both the vector and packaging constructs can reduce the probability of recombination and insertional mutagenesis events. Splitting the packaging sequences between as many constructs as possible and careful sequence manipulation to reduce homology between those constructs will significantly reduce the likelihood of recombination events giving rise to replication competent viruses.

The use of packaging cell lines stably expressing the packaging sequences will also reduce the likelihood of recombination resulting in the production of replication competent viruses. Cotransfection methodologies bring high-levels of plasmid DNA together within cells and therefore increase the probability of DNA homologous recombination giving rise to a competent viral genome. Cell lines that have been screened for endogenous proviruses will reduce the likelihood of recombination events and mobilisation of endogenous proviruses by superinfection with the vector.

Consideration of the ability of a GMO to establish an infection *in vivo*

An assessment should be made as to the ability of the GMO to establish an infection, how efficient that infection would be and its ability to spread within the host or within a community. This represents an evaluation of the 'fitness' of a GMO and should be based upon established scientific knowledge rather than assumptions, where possible. If the gene therapy vector is attenuated, it may not be infectious even if it can enter target cells.

It is important that fitness is not coupled with pathogenicity per se as some modifications whilst theoretically making the GMO more pathogenic may also render the GMO less fit. For example, consider the insertion of a foreign gene into the E3 locus of adenovirus. The modified virus will be less likely to establish an infection and spread in the community as the loss of E3 makes the virus more susceptible to immune surveillance and therefore reduces the risk of environmental spread.

For many gene therapy vectors it should be acknowledged that there is widespread immunity in the human population, which is likely to reduce the ability of the GMO to spread into the community. For example, in Europe, most people will have immunity to adenovirus serotype 5, although young children may be more susceptible. The level of immunity in the community may be an important consideration in the ERA, but the data need to be carefully considered, particularly in relation to vulnerable groups.

Consideration of the probability that rare events will occur

It is may be possible to assign a frequency to a given event. Often, this can take the form of a precise numerical frequency obtained in-house or through published data. For example, the rates of mutation and frequencies of recombination during viral replication are open to quantitative analysis and are known and published for many viruses.

In many cases, however, this will not be possible and an approximate, semi-quantitative or descriptive assessment of the frequency, based upon experience with similar GMOs or techniques can be used. For example, the likelihood of an attenuated or disabled GMO reverting to wild type status can be

assessed on the basis of the number of discrete events that would need to take place, i.e. the more events needed, the less likely it is that reversion will occur.

It should not be assumed, however, that failure to observe or detect an event is evidence that it does not occur. As part of such considerations it should be recognised that micro-organisms often have extremely short generation times and therefore adapt to specific environments and selective pressures rapidly. This is particularly true for viruses and during the course of evolution they have proved particularly adept at responding to selective pressures by infecting new cell types or host organisms.

For each potential adverse effect, conditions of administration to the patient (dose, route of administration) and conditions of potential exposure of the environment (magnitude, duration) should be evaluated.

The route of administration of the GMO will influence environmental exposure. In general the exposure will be higher if for instance, the GMO is administered via a nasal spray than if the GMO is administered orally or by injection. The site and mode of injection (e.g. intramuscular, intravenous) will influence systemic distribution, and therefore shedding.

The magnitude of the exposure depends on characteristics of the surrounding environment, including medical staff members, social contacts and the general public. Procedures that might lead to exposure, include i) production and preparation of the GMO; ii) administration and iii) waste disposal. Shedding is an important factor in the exposure of the environment to the GMO, but is not in itself an adverse effect. Increased shedding resulting in greater environmental exposure only leads to a high risk if there are significant consequences, as identified earlier in the ERA.

An example could be a replication-incompetent viral vectors carrying a transgene used to treat bladder cancer. Shedding/excretion of the product in urine could be significant, whereas the environmental risk could be negligible as the virus is unable to replicate. In the case of a replicating virus this may be quite different. Furthermore, lytic viruses such as herpes and vaccinia may cause surface lesions, leading to viral shedding. This may be managed by using attenuated non-lytic derivatives, or through management of the lesion, such as the use of appropriate dressings. Such issues need to be considered in the ERA, and an appropriate strategy adopted.

The likelihood can best be described in qualitative terms ranging from high, moderate, low to negligible as the consequences of potential adverse effects in step 2.

The following issues should be addressed:

a. The intended use: clinical sites that are expected to administer the medicinal product; details of the route and mode of administration; possibility of off-label use / named patient / use outside of the approved indication
b. Circumstances of previous clinical use
c. Relevant details of previous deliberate release / contained use / marketing authorisations or injunctions
d. History of and changes to precautions, including history of accidents and results of risk managing measures.
e. Adverse effects or events related to ,or with implications for, the environmental risk.
f. Biodistribution: presence in bodily fluids, secretion, shedding, transmission via mucosal surfaces, via blood.

Testing methods. PCR, a sensitive detection method, will only detect the presence of GMO genomes without giving any information about the infectivity of the vector particle. Typically, the result will be the same before and after inactivation. More traditional culturing methods give an indication of the infectious vector titre but are time-consuming. Such culturing may be difficult for replication incompetent GMOs. Combinations between culturing and PCR detection may yield an improved rate of detection. Extensive testing may not be necessary if the risk is found to be negligible.

Step 4. **Estimation of the risk posed by each identified characteristic of the GMO(s): An estimation of the risk to human health or the environment posed by each identified**

characteristic of the GMO which has the potential to cause adverse effects should be made as far as possible, given the state of the art, by combining the likelihood of the adverse effect occurring and the magnitude of the consequences, if it occurs.

To estimate the environmental risk, the magnitude of the consequences of the identified potential adverse effects is combined with the outcome of the evaluation of the likelihood. The risk may also be described in qualitative terms ranging from high, moderate, and low to negligible. While it is not possible to multiply qualitative terms, a risk matrix is useful as a tool that illustrates the process of risk estimation.

Step 5. **Application of management strategies for risks from the deliberate release or marketing of GMO(s): The risk assessment may identify risks that require management and how best to manage them, and a risk management strategy should be defined.**

In a clinical trial situation risk management (e.g. containment of patients) is potentially more easily achievable, compared to that after a MA has been granted, and the use of the medicinal product is no longer fully controlled by the applicant. Therefore, in the interest of a smooth transition from the clinical trial stages to a marketing authorization, it may be prudent to start collecting data on the feasibility or (un)necessity of implementing risk management strategies at an early stage.

Precautions can be implemented to decrease an environmental risk. Risk management strategies may affect i) the characterised hazard, ii) the consequences of the hazard occurring or iii) the estimated likelihood. In most cases the likelihood is reduced. Some examples of risk management measures are:

i) Specification of contra-indications, e.g. acute viral infections or a compromised immune status;

ii) Requirement for hospitalisation and hygienic measures. Isolation effectively reduces environmental risks but creates a problem if a patient decides to leave the hospital early. Furthermore, the patient population of a hospital may be more than normally vulnerable to the GMO making it important to prevent infection and environmental spread in the hospital.

iii) In cases of parenteral administration leakage can be reduced by sealing the injection site.

iv) Control measures to minimise aerosol formation: adenoviruses are robust and transmitted effectively in aerosols and droplets, even if disabled or attenuated.

The effectiveness of risk management strategies can be evaluated by identifying the step in the ERA that is affected by the risk management. Precautionary measures should decrease estimated risk but be proportional to the seriousness of the risk. Some remaining uncertainty is inevitable. Medicinal products are subject to post-registration monitoring and such pharmacovigilance may contribute to environmental risk management. New data emerging from this monitoring should be evaluated for their impact on environmental safety. Management measures to reduce the risk to an acceptable level should be described in the SPC. When unacceptably invasive risk management strategies are required to reduce the risk to the environment to an acceptable level, the conclusion of the overall risk assessment may have a negative impact on the overall benefit/risk analysis for the medicinal product.

Management or monitoring procedures that are in place for purely medical reasons should not be listed as environmental risk management procedures. A mix up of these procedures might legally require an applicant to continue medical monitoring even when it is no longer necessary from a medical point of view.

Demonstrating replication incompetence

It is good practice to demonstrate that vector stocks are devoid of replication competent vector. Moreover, this should be mandatory where the risk assessment is based on the assumption that replication competent vector is absent. Direct plating of vector stock onto permissive cell lines and observation of indications of viral replication (for example cytopathic effect or syncitia formation)

could be used to detect replication competent vector particles; however such approaches do not always give a clear result and specific molecular detection methods could also be employed. For example viral protein expression could be detected by immunostaining or gag, pol or env DNA proviral sequences could be detected using PCR.

A means for monitoring the generation of RCV should be in place where appropriate. Disabled GM virus stocks tested on permissive, non-complementing cell lines should show signs of productive infection (cytopathic effect, plaque formation) in the presence of RCV. Such assays may not be completely reliable, however, as cytopathology is often evident even with disabled viruses. The use of molecular detection, for example quantitation of E1 sequences in a purified virus preparation using quantitative PCR, would represent a more reliable method.

Emergency plan. Arrangements for emergencies arising during transport to treatment centre(s), transport to treatment site(s) within centre(s); handling of the GMO-containing product at the treatment centre, accidental skin contact, accidental eye contact and accidental needle stick injuries. Prevention methods for infection, treatment and prevention methods for disease/availability of vaccines

SOPs and emergency plan. Several aspects of the use of a GMO may require SOPs or an emergency plan, e.g. waste disposal or needle stick accidents. It may not be feasible to prescribe standard operating procedures for individual hospitals in different member states. The ERA should indicate if special measures, outside standard clinical procedures (e.g. sharps bins) are required to keep environmental risks at an acceptable level. Where necessary, guidance should be provided in the SPC for dealing with environmental safety and safety for medical staff and other contacts during use of the GMO.

Waste treatment. It may be necessary to use disinfectants and subsequent waste treatment. Environmental issues that result from the use of the disinfectant itself are not part of this ERA. It should be noted that disinfectants themselves are also subject to licensing for use in individual member states so it may be necessary to determine if the disinfectant of choice is actually permitted in the concerned member state. The following should also be considered: disinfection of materials used for transport, preparation or administration; including surfaces, instruments, hoods, clothing, and gloves, efficacy of the disinfection methods or agents proposed, measures taken following a spill.

Post marketing monitoring. The following should also be considered: expected effects on other humans; on animals and/or plants; on the environment; conditions for release of treated patients; monitoring plan or reasons for not installing a monitoring plan; monitoring of methods installed to prevent spread to medical personnel or people in the environment.

Step 6. Determination of the overall risk of the GMO(s): An evaluation of the overall risk of the GMO(s) should be made taking into account any risk management strategies which are proposed.

Based on the previous steps a conclusion should be given as to whether the overall environmental impact is acceptable or not. This final evaluation should be expressed as a summary of the overall risks that are connected to the specific gene therapy application.

DEFINITIONS

Environmental risk. In the framework of medicinal products, mainly the risk of transmission of the GMO to humans other than the intended person or patient, to animals or to the environment at large. A secondary risk may be posed by pathogens, which arise by recombination with the original GMO.

Monitoring plan. Following deliberate release into the environment, directive 2001/18 requires that "general surveillance" and "monitoring as appropriate" are performed. A monitoring plan should include systemic distribution and shedding (if shedding data must be included also depends on the outcome of the environmental risk assessment), surveillance of long term side effects, information about adverse or unexpected effects, monitoring effects in others than the patient and information

about off label use. An overview of sampling strategy and analytical methods to be used should be given.

REFERENCES (SCIENTIFIC AND / OR LEGAL)

• Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms.

• Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use.

• Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency.

• Communication from the Commission on the precautionary principle, COM (2000) 1 final, dated 2.02.2000. http://ec.europa.eu/environment/docum/20001_en.htm

• Council Decision of 3 October 2002 establishing guidance notes supplementing Annex VII to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.

• Council Decision of 3 October 2002 establishing pursuant to Directive 2001/18/EC of the European Parliament and of the Council the summary information format relating to the placing on the market of genetically modified organisms as or in products.

Note: See http://publications.eu.int/code/en/en-250304.htm for guidance on referencing published information and http://publications.eu.int/code/en/en-130102.htm for guidance on referencing EU texts. References to related guidelines should also be included.