Good Practice on the assessment of GMO-related aspects in the context of clinical trials with human cells genetically modified by means of retro/lentiviral vectors[1]

VERSION 3

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Version 1	July 2018	
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[1] This document has not been adopted by the European Commission and, therefore, it does not contain the official position of the European Commission.

1. Introduction

Clinical trials conducted in the EU with investigational medicinal products that contain or consist of genetically modified organisms ("GMOs"[2]) must comply with the legislation governing the authorization of clinical trials.[3] The authorization procedure under the clinical trials framework aims to ensure the rights, safety, dignity and well-being of those individuals that take part in a clinical trial as well as the reliability and robustness of the data generated.

Clinical trials with medicinal products that contain or consist of GMOs must also comply with applicable requirements under Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms[4] ("deliberate release framework") and/or under Directive 2009/41/EC on the contained use of genetically modified micro-organisms ("contained use framework").[5] The GMO regulatory framework aims to ensure a high level of protection for human health and the environment.

Recent discoveries in biomedicine have created the expectation that gene therapy medicinal products can give responses to some of today's unmet medical needs, or provide novel solutions to diseases such as cancer or neurodegenerative disorders. Gene therapy medicinal products cover a wide range of products with different levels of risks for human health and the environment. A need has been identified for guidance on the application of the GMO framework to human cells genetically modified by means of retro/lentiviral vectors when used in a clinical trial setting. Such guidance should take into consideration the specific characteristics of the concerned investigational medicinal products and the risks thereof to public health and the environment.

This Good Practice document has been jointly developed by the European Commission services[6] and the competent authorities of the Member States responsible for the implementation of the legislation on clinical trials and those responsible for the implementation of the GMO legislation having regard to accumulated experience with this type of medicinal products. The document builds on the principles expressed by Council of the European Union on research and innovation policies and, specifically, regarding the use of

[2] Throughout this document, the term "GMO" should be understood as covering both genetically modified organisms as defined under Article 2(2) of Directive 2001/18/EC, and genetically modified micro-organisms within the meaning of Article 2(b) of Directive 2009/41/EC.

[3] Regulation (EU) No 536/2014 on clinical trials of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use and repealing Directive 2001/20/EC, (OJ L158, 27.5.2014, p.1). Until the Regulation enters into force, Directive 2001/20/EC applies (Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use, OJ L121,1.5.2001, p.34).

[4] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (OJ L 106, 17.4.2001, p. 1).

[5] Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms (OJ L 125, 21.5.2009, p. 75).

[6] This document is one of the outputs from the dialogue with national competent authorities to address the interplay between the GMO and the medicines legislation as foreseen in the Joint European Commission-DG Health and Food Safety and European Medicines Agency Action Plan on ATMPs.

all possibilities under the existing legislation to facilitate investments in research and innovation and that to this end Member States should also consider reviewing their own national frameworks and implementation of EU law.[7]

The document has been endorsed by the national competent authorities of the following Member States: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Luxembourg, Malta, the Netherlands, Portugal, Romania, Spain and Sweden. The document has also been endorsed by Norway.

The Good Practice document is to be used in conjunction with the common application form specifically developed for this type of investigational medicinal product in all of the above referred countries.

2. Scope

This document provides guidance on the implementation of the regulatory requirements under the GMO framework applicable to the conduct of clinical trials with human cells genetically modified by means of retro/lentiviral vectors in cases where the applicant demonstrates that:

(i) there is no or negligible risk of formation of replication competent virus in accordance with Section 3(1), and

(ii) the finished product is free of residual infectious viral vector particles that are capable of being released in the environment in accordance with Section 3(2).

The concerned investigational medicinal product contains a stably integrated construct expressing one or more donor genes. The donor genes may be of different origin (human, viral, bacterial, *etc.*).

For the purposes of this document, retroviral vector means murine gamma-retroviral vectors. In connection with lentiviral vectors, this document has been developed on the basis of knowledge derived from human cells transduced with lentiviral vectors derived from HIV virus. In case of lentiviral vectors derived from other viruses, developers are invited to do a risk assessment and contact the relevant competent authority.

The requirements laid down in this document are applicable to cases where the conduct of the clinical trial is regulated under the deliberate release framework and also where conduct of the clinical trial is regulated under the contained use framework.

It is stressed that the content of this document (including the specific environmental risk assessment ("ERA")) cannot be extrapolated to products other than human cells genetically modified by means of retro/lentiviral vectors in cases where the applicant demonstrates absence of formation of replication competent virus and absence of residual infectious viral vector particles in the transduced cells in accordance with Section 3(1) and 3(2).

[7] Council conclusions on research and innovation friendly regulation adopted by the Council at its 3470th meeting held on 27 May 2016.

3. Environmental risk assessment and data requirements

Human cells cannot proliferate in the environment as they can only survive inside the human body or under *in vitro* culture conditions. It follows that, when the investigational medicinal product consist of human cells genetically modified by means of retro/lentiviral vectors, the risks to the environment and public health are mainly linked to the potential for formation of a replication competent virus and to the presence of residual infectious viral vector particles in the finished product that could be released in the environment.

Having regard to the above, as well as the accumulated experience with the assessment of human cells genetically modified by means of retroviral and lentiviral vectors, the assessment of applications for the conduct of clinical trials with investigational medicinal products covered by the scope of this document should be done on the basis of the description of the viral vector used, the evidence submitted to demonstrate absence of formation of replication competent virus, and the evidence submitted to demonstrate absence of residual infectious viral vector particles in the investigational medicinal product. To this effect, the competent authorities responsible for the application of the GMO framework in Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany Greece, Hungary, Ireland, Italy, Latvia, Luxembourg, Malta, the Netherlands, Portugal, Romania, Spain, Sweden, and Norway have agreed a common application form which can be used to seek authorisation under the GMO framework for the conduct of the clinical trial with investigational medicinal products covered by the scope of this document.

In addition, it is considered that the conduct of clinical trials with the investigational medicinal products covered by the scope of this document entails no risks to public health or the environment. Therefore, for the purposes of the environmental risk assessment of the concerned investigational medicinal product, applicants can refer to the specific ERA provided in the Annex to this document. [8]

3.1. Demonstration of absence of formation of replication competent virus

The risk of recombination of the constituent parts of the viral vector system with each other, or with cellular sequences, which can generate a replication competent retrovirus/lentivirus should be minimised. In particular, applicants are expected to address the following aspects:

- The retro/lentiviral production system is a retro/lentiviral system that is devoid of sequences required for RCR/RCL formation. [9]

- The applied production cell-line does not contain HIV-1, HIV-2, HTLV-1, HTLV-2, SIV or other relevant retro/lentiviruses that could lead to complementation and/or recombination of the retro/lentiviral vector.

[8] An ERA is not required for applications submitted under contained use framework.

[9] Current examples are the 2nd generation self-inactivating (SIN) system, the 3rd generation SIN system, and the 4th generation translentiviral systems.

- The retro/lentiviral batch used for transduction is tested for the presence of replication competent virus with a validated test at the level of the viral production system or, alternatively, in the transduced cells (each batch of the finished product should be tested in cases where there has not been testing at the level of the viral production system).

- The applied insert(s) does not lead to complementation of the retro/lentiviral vector.

3.2. Demonstration of absence of residual infectious viral vector particles in the transduced cells.

Applicants should demonstrate that residual infectious retro/lentiviral particles have been reduced to negligible concentrations. There may be more than one way to demonstrate this, including qualitative or quantitative methods. The formula provided in Table 1 can be used but other methods are also acceptable.

4. Manufacturing requirements and containment levels

The manufacturing of the investigational medicinal products covered by the scope of this document should be done under appropriate conditions. To this effect, the manufacturing of viral vectors and the *ex vivo* transduction of human cells with viral vectors should be regulated under the contained use framework. It is recalled that manufacturing of investigational medicinal products (including genetically modified human cells) should be in accordance with Good Manufacturing Practice.[10]

The biosafety level of these activities should be determined according to the specific characteristics of the vector system. In determining the applicable biosafety level ("BSL level") the following considerations apply:

i) Most manufacturing activities with cells involving lentiviral systems (2nd and 3rd generation systems and the 4th generation translentiviral systems) and (mouse gamma-) retroviral systems can be carried out under BSL-2 conditions.

ii) The transduction of the cells should be done under BSL-2 conditions.

iii) Other down-stream manufacturing activities (*i.e.* after transduction) can, however, be downgraded to BSL 1, when all the conditions laid down in the table below are met.

Table 1- Criteria for downgrading the biosafety level to BSL-1

Criteria	**Conditions (cumulative)**
Molecular characterization of the	• Full characterisation (*i.e.* full sequence) of the viral vector used for cell transduction, and characterization

[10] https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2017_11_22_guidelines_gmp_for_atmps.pdf

applied vectors	of the critical elements on helper/packaging vectors. • It should be shown that there are no deviations from the predicted sequences.
Absence of formation of replication competent virus in the viral production system	• The applied retro/lentiviral production system should be a retro/lentiviral system that is devoid of sequences required for RCR/RCL formation.[11] • The applied production cell-line should not contain HIV-1, HIV-2, HTLV-1, HTLV-2, SIV or other relevant retro/lentiviruses that could lead to complementation/recombination of the retro/lentiviral vector. • The retro/lentiviral batch used for transduction is tested for the presence of replication competent virus with a validated test. • The applied insert(s) should not lead to complementation of the retro/lentiviral vector.
Absence of replication competent virus in the GM cells	• Cells from HIV and HTLV positive patients/donors are excluded. • The transduced cells have been tested for the presence of replication competent retro/lentivirus with a validated test, unless appropriate justification is provided by the applicant (*e.g.* absence of formation of replication competent virus has been demonstrated at the level of the viral production system).
After transduction, the GM cells must be free of residual infectious viral particles	Residual infectious retro/lentiviral particles have been reduced to negligible concentrations. There may be more than one way to demonstrate this, including qualitative or quantitative methods. For example, the formula below[12] can be applied to determine the so-called "reduction ratio" to allow downscaling from BSL-2 to BSL-1 (other alternative calculations that may be submitted by the applicant may also be acceptable): $\boldsymbol{Reduction\ ratio = (20^{W} \times 200^{I} \times 2^{2,4T})/C^{i}}$ *In this formula:*

[11] Current examples are the 2nd generation self-inactivating (SIN) system, the 3rd generation SIN system, and the 4th generation translentiviral systems.

[12] This formula is based on a formula developed by the Netherlands Commission on Genetic Modification (COGEM).

	W *is the number of washing steps (assuming that each washing step leads to a 20-fold*[13] *reduction of the amount of viral particles),* ***I*** *is the number of inactivating washing steps with trypsine or human serum (assuming that each inactivating washing step leads to a 200-fold*[14] *reduction of the amount of viral particles and may be adjusted when a different envelope than the VSV-G envelope is applied for pseudotyping)* ***T*** *is the culture time in days after transduction.* *The factor 2,4 in the formula is based on the halftime (10 hours) of VSV-G pseudotyped lentiviral vectors at a temperature of 37°C and has to be adjusted when a different envelope than the VSV-G envelope is applied for pseudotyping.* [15] $\boldsymbol{C^i}$ *is the initial viral titer applied in the inoculum.* *A resulting reduction ratio of >100 (two logs) may be accepted for downscaling retro/lentivirally transduced cells from BSL-2 to BSL-1.*
The viral sequences in the GM cells cannot be reconstituted to form new viral particles	Cells are cultured under conditions to prevent (re-)infection with lentiviruses or retroviruses from other sources during culture.

[13] A different reduction level may be applied if this supported by validated data from the applicant.

[14] A different reduction level may be applied if this supported by validated data from the applicant.

[15] A different factor than 2,4 may be applied if supported by validated data from the applicant.

Annex – Specific ERA

1. Scope

This specific environmental risk assessment can be applied to investigational medicinal products which meet the following cumulative conditions:

i) the investigational medicinal product consist of human cells genetically modified by means of retro/lentiviral vectors,

ii) the applicant has demonstrated that there is no risk of formation of replication competent virus and that the finished product is free of infectious viral vector particles that are capable of being released in the environment in accordance with Section 3, and

iii) the finished product is intended as a medicinal product for humans only and is administered in clinical centres in the context of an authorised clinical trial.

Throughout this document, the term "concerned investigational medicinal product" is used to refer to a product that meets the three above-referred conditions.

2. General considerations

Human cells cannot proliferate in the environment as they can only survive inside the human body or under *in vitro* culture conditions.

The expression of donor genes is highly unlikely to alter the survival of human cells in the environment but may alter cellular behaviour, *e.g.* cell cycle regulation, apoptosis, proliferation and survival under *in vitro* culture conditions or in the human body.

It follows that potential hazards of the clinical use of the concerned investigational medicinal product are therefore only related to human health. Potential hazards to animal health or the environment are not applicable.

3.1. Identification and characterization of hazards.

There are no hazards to animal health or the environment.

Potential hazards to human health

Hazards related to the persistence of the genetically modified human cells in the population

The concerned investigational medicinal product is administered to patients with a view to treat, prevent or cure an underlying condition. The persistence of the concerned investigational medicinal product in the body of the treated patient does not constitute a hazard to the human health. However, while unlikely, a potential hazard could exist if there was an unintended transfer of the concerned investigational medicinal product to individuals other than the targeted patient.

Human cells have no colonization ability in immune-competent individuals. Even if the presence of the integrated lentiviral vector or retroviral vector constructs or expression of the donor sequence has an influence on the phenotypic characteristics of the genetically modified cells, this does not confer any specific competitive advantage to the genetically modified cells in immune-competent individuals. Therefore, in case of accidental transfer of the concerned investigational medicinal product to non-target human subjects, the genetically modified cells would be cleared by the immune system of a healthy individual.

In case of unintentional transfer (*e.g.* accidental transfer to healthcare professional or administration error to a different patient) to immune-incompetent individuals, potential hazards could, in theory, exist. The potential consequences of such an accidental transfer would depend on the effects of the integrated construct and the expressed donor gene sequences on the phenotype of the target cell.

Hazards associated with recombination or mobilization of viral sequences from the integrated construct

Potential hazards could occur if there was mobilization or recombination of integrated lentiviral vector or retroviral vector constructs upon infection of the transplanted cells with HIV or retroviruses in the patients with an active infection. Another scenario of potential hazard would be the recombination of donor genes of viral origin present in the lentiviral vector or retroviral vector construct with related endogenous viruses upon infection of the transplanted cells with a highly related virus, leading to a novel GM virus (provided that the recombination allowed replication of the recombined sequences).

The potential consequences of such an event would depend on the characteristics of the novel formed GM virus but could potentially lead to harmful effects in thirds in case of horizontal transmission.

3.2. Exposure characterisation

Likelihood of adverse effects linked to the persistence of genetically modified human cells in the population

Three possible scenarios could be envisaged: [16]

(1) Accidental transfer to immune incompetent individuals: A possible scenario of accidental transfer to thirds would be in case of a needle-stick accident during administration. In such a case, transfer of the genetically modified cells to the accidental recipient of the product could take place. However, this would only lead to persistence in case of an immunodeficiency of the accidental recipient of the product, since the probability that both the patient and the accidentally injected person have the same MHC-haplotypes is negligible.

[16] The scenario of contamination through the environment (bleeding of the patient) is not considered realistic given that cells cannot survive outside the human body.

While transduced human cells may persist in the human body of an immune-incompetent individual and this persistence may be long-lasting, depending on the characteristics of the applied cells, the likelihood of harmful effect in case of accidental transfer is considered negligible given (1) the absence of replication competent and infectious viral vector particles in the transduced cells, (2) the low numbers of cells that would be introduced in the case of an accidental transfer, and (3) the absence of preconditioning regime in the accidental recipient of the cells.

(2) Erroneous administration to a different patient. As in the scenario above, the transduced cells would only persist if the patient that received the concerned investigational medicinal product due to an administration error was immunodeficient.

The likelihood of harmful effect in this scenario is low given the absence of viral vector particles in the transduced cells. Moreover, it is extremely unlikely that the accidental recipient in this scenario would have been subject to a preconditioning regime that would support the long-term survival of the transduced cells.

(3) Donation of blood, cells, tissues or organs to immune-incompetent thirds. A possible scenario of transfer to thirds would be in case of transfusion of blood or transplantation of cells, tissues or organs from a donor that has been treated with the concerned investigational medicinal product.

The likelihood of harmful effect if transduced cells from a patient treated with the concerned investigational medicinal product are transferred to a third party *via* donation is considered low given the absence of viral vector particles in the transduced cells.

Moreover, the concerned investigational medicinal product is often administered to treat conditions which, *de facto*, imply the non-eligibility of the patient as a future donor.[17] In such cases, the likelihood of harmful effect is negligible.

Likelihood of mobilization or recombination of the viral vector with other viruses

The mobilization or recombination of the viral vector with other virus is generally considered unlikely due to the deficient structure of the vectors commonly used for the manufacture of gene therapy medicinal products.

[17] Eligibility criteria for donors of whole blood and blood components are laid down in Annex III to *Commission Directive 2004/33/EC of 22 March 2004 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components* (OJ L91, 30.3.2004, p. 25), as amended.
Selection criteria for donors of tissues and cells are laid down in Annex I to *Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells* (OJ L38, 9.2.2006, p. 40), as amended.
Organ and donor characterisation criteria for organs are laid down in the Annex to *Directive 2010/45/EU of the European Parliament and of the Council of 7 July 2010 on standards of quality and safety of human organs intended for transplantation* (OJ L207, 6.8.2010, p. 14).

In connection with the scenario of mobilization of the integrated lentiviral or retroviral construct upon infection of donor cells in the patient's body with HIV, HTLV or endogenous retroviruses, it is noted that, while the possibility of mobilization and recombination has been identified under *in-vitro* conditions, it has never been observed in clinical trials conducted in the past 20 years, including in clinical trials with HIV patients.

The likelihood of mobilization of the integrated lentiviral construct upon infection of donor cells in the patient's body with HIV, HTLV or endogenous retroviruses is considered to be negligible for the following reasons:

- In the case of lentiviral vectors, the replication-defective nature of the vector prevents the possibility of spontaneous mobilization of the integrated vector from the transduced cells unless helper functions are provided in the transduced cells by super-infection with wild-type virus in an infected host. The self-inactivating feature (SIN) of the vector LTR prevents vector mobilization even in the case of superinfection of the transduced cell by a wild-type virus.

- In the case of gamma-retroviral vectors, a recombination event is also highly unlikely considering that, in principle, the murine retroviruses do not infect humans. The risk of provirus or free vector to be mobilised through recombination between the integrated vector genome and genetic sequences from potentially co-infecting retroviruses is a very theoretic risk. Exogenous, infectious gamma-retroviral viruses have not been found in humans and recombination between vector sequences and non-gamma-retroviral co-infecting retroviruses would not be expected to produce an RCR. In order for a provirus to become an RCR it would need to obtain both heterologous gag-pol and env coding sequences from other sources within the same cell as the provirus. There are no known exogenous gamma-retroviruses in human populations which could introduce functional gag-pol and env coding sequences to transduced cells.

3.3. Risk characterisation

Risk associated with the persistence of genetically modified human cells in the population

The only possible risks are associated with the unintended transfer of donor cells to immune-incompetent individuals in three possible scenarios:

(1) Accidental transfer to immune incompetent individuals: For the reasons explained above, the magnitude of adverse effects linked to accidental transfer of the concerned investigational medicinal product to immune incompetent individuals is negligible. In addition, the concerned investigational medicinal product is administered by trained professionals in a highly controlled environment, which minimizes the probability that an accidental transfers can occur during the administration/handling of the product. Moreover, the probability that an accident occurs during administration/handling of the product and that the healthcare professional affected is also immune incompetent is

considered extremely low. Therefore, it can be concluded that the risk associated with the persistence of the genetically modified cells in the scenario of accidental transfer is negligible.

(2) Erroneous administration to a non-intended patient. For the reasons explained above, the magnitude of adverse effects linked to accidental transfer of the concerned investigational medicinal product to immune incompetent individuals is low. Additionally, the administration to patients of the concerned investigational medicinal product takes place by trained personnel in a highly controlled environment which includes strict labelling and traceability requirements to avoid administration errors. It follows that the risks to immune-incompetent individuals in the scenario of accidental transfer are negligible.

(3) Donation of blood, cells, tissues or organs to immune-incompetent thirds: As explained above, the probability of adverse effects linked to accidental transfer of the concerned investigational medicinal product to immune incompetent individuals is low. In cases where the genetically modified human cells are intended to treat conditions which disqualify the patient as potential donor, the risks to immune-incompetent individuals via transfusion/transplant are negligible. In other cases, the applicant should consider if patients should be prevented from donating blood/cells/tissues/organs after being administered the genetically modified human cells.

Risks associated with recombination of mobilization of viral sequences from the integrated construct

The only possible risks are associated with the mobilization of integrated lentiviral sequences upon active infection of the donor cells with HIV or HTLV.

As explained above, the probability of mobilization or recombination of the viral vector with other viruses is negligible. Therefore, the risks for human population would be negligible.

3.4. Risk management strategies

The applicant should consider if patients should be prevented from donating blood/cells/tissues/organs after being administered the concerned investigational medicinal product. In the case of submissions in jurisdictions that apply the common application form, this should be explained in Section 3 thereof.

Adequate measures should be implemented to prevent risks of accidental transfer during administration to health care professionals involved in the handling/administering the product. For submissions in jurisdictions that apply the common application form, this should be explained in Section 3 thereof.

Adequate measures should be in place for storage, transportation and waste treatment. For submissions in jurisdictions that apply the common application form, this should be explained in Section 3 thereof.

3.5. Determination of the overall risk and conclusions

No risks to the environment or animal health can be identified. Provided that the control measures described by the applicant (in the case of submissions in jurisdictions that apply the common application form, the control measures are described in Section 3) are implemented, the overall risks for human health are considered negligible.