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To the Minister for the Environment and Public Transport
Ministery of Infrastructure and Water Management
Mr.A. Aartsen
P.O. Box 20901
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DATE 27 November 2025
REFERENCE CGM/251127-01
SUBJECT Update of prerequisites for clinical studies involving transduced cells

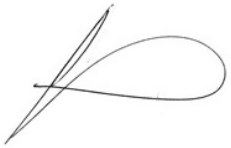
Dear Mr Aartsen,

Several years ago, COGEM published a generic environmental risk assessment for clinical studies using genetically modified cells. The assessment outlined conditions to which these clinical studies should adhere to result in a negligible environmental risk. These prerequisites also simplify the licensing procedure for these studies. Over time, these prerequisites have been tightened or amended. The current advisory report provides an overview of the updated prerequisites.

Summary:
In 2019 and 2020, COGEM conducted generic environmental risk assessments for clinical studies involving cells that have been modified outside the body (ex vivo) using retroviral and lentiviral vectors. These assessments established conditions that clinical studies must meet to ensure that environmental risks are negligible. These assessments also contributed to the drafting of permits under fixed conditions ('vergunningen onder vaste voorwaarden', VoVs) in the Netherlands. This allows such studies to be processed more quickly and easily.
Following the initial assessments, COGEM issued additional recommendations. Some prerequisites were tightened or amended in line with new insights.
The current advice updates the previous generic environmental risk assessments. It summarises and explains the current prerequisites for clinical studies involving the use of ex vivo retro- and lentiviral transduced cells. COGEM considers the risks to humans and the environment in such clinical studies to be negligible if these prerequisites are met. COGEM recommends incorporating these updated prerequisites into the VoVs.

The attached report contains COGEM's advice and a discussion of the underlying reasoning.

Yours sincerely,



Professor Sybe Schaap
Voorzitter COGEM

c.c.

- Drs. Y. de Keulenaar, head of the GMO Office
- Environmental Safety and Risks Directorate, Directorate-General for the Environment and International Affairs, Ministry of Infrastructure and the Environment
- Dr. M.M.C. Gielkens, Gene Therapy Office

Advice Update on prerequisites for generic environmental risk assessment of clinical studies and marketing applications involving ex vivo retro- and lentiviral transduced cells

COGEM-advice CGM/251127-01

1. Introduction

In 2019 and 2020, COGEM published two generic environmental risk assessments: one focusing on clinical studies involving ex vivo retro- and lentiviral transduced cells in the absence of free vector particles[1] and another on ex vivo lentiviral transduced cells in the presence of free lentiviral vector particles in the medical product.[2] These assessments listed several prerequisites that such studies must meet to achieve a negligible risk to third parties and the environment. Based on new insights, COGEM subsequently recommended several refinements and changes to the prerequisites.[3,4,5]

This advice provides an update of the previous environmental risk assessments and provides an overview of COGEM's current recommended prerequisites for clinical studies involving ex vivo retro- and lentiviral transduced cells.

2. The generic environmental risk assessment of clinical studies involving ex vivo transduced cells

COGEM regularly advises on clinical studies in which patient-owned or donor cells are transduced ex vivo using retroviral or lentiviral vectors. This process results in the integration and expression of a transgene in the genome of these cells. After transduction, the genetically modified (GM) cells are returned to the patient via an infusion.

The generic environmental risk assessment of such clinical studies is limited to the use of retroviral vectors based on the Moloney murine leukaemia virus (MoMuLV), or self-inactivating (SIN) vectors derived from human immunodeficiency virus 1 (HIV-1) and produced using a third-generation production system. These are the most commonly used vectors in clinical studies involving ex vivo transduced cells, and much has been learned and published in the scientific literature about the biosafety of these vectors.

The following aspects are important for the environmental risk assessment of clinical studies involving these vectors: (i) the potential formation of replication-competent viruses during production of viral vectors, (ii) the presence of free vector particles in the medical product, and (iii) the possible recombination or complementation of the vector in the medical product.

2.1 Possible formation of replication-competent virus

One risk associated with the production of viral vector particles is the emergence of replication-competent virus (RCV). RCVs can arise through recombination between the plasmids necessary for producing the viral vector. In the case of retroviral vectors, they can also arise through recombination with endogenous retrovirus sequences present in the genome of the cell line used. With regard to retroviral vectors, COGEM has previously stated that the risk of replication-competent retrovirus (RCR) arising during production cannot be entirely ruled out.[1] COGEM therefore considers it necessary to use

a validated test to rule out the presence of RCRs in the vector batch, the end-of-production (EOP) cells, or the final medical product (the transduced cells).

There have never been any reports in the scientific literature of the emergence of replication-competent lentivirus (RCL) when using lentiviral SIN vectors produced with a third-generation production system. At least three different recombinations must occur during the production of third-generation lentiviral SIN vectors, and the deletion of the promoter sequence in the LTR of the SIN construct must be compensated for before RCL can arise. Therefore, COGEM has previously stated that the formation of RCLs during the production of third-generation SIN lentiviral vectors is impossible, and testing for their presence during production is unnecessary.

2.2 Presence of free vector particles

After transduction of the cells, free infectious vector particles may remain in the medical product being tested. These free vector particles are considered a risk factor because spread to third parties (such as family members) could lead to unintended transduction. This could result in the undesirable expression of the transgene in the recipient's cells. Additionally, it cannot be ruled out that the integration of the vector genome could disrupt healthy cells, which, in the worst-case scenario, could result in cancer. The number of retro- or lentiviral vector particles transferred during unintended exposure is an important factor in environmental risk assessments. The number of free vector particles can be determined experimentally. If this is not possible, the “COGEM formula” can be used instead.[6,7,8] COGEM recently issued an advice providing an update and further explanation of the formula.[8]

As part of the prerequisites for clinical studies involving ex vivo retro- and lentiviral transduced cells, COGEM has recommended a limit value for vector particles. If this limit is exceeded, several generic control measures must be implemented to minimise the risk of spreading lentiviral and retroviral vector particles (see section 4). COGEM has recently adjusted this limit value to 500 free vector particles (equivalent to a reduction ratio of 0.002), which applies to both retroviral and lentiviral vector particles.[9]

2.3 Recombination or complementation of the vector

Previously, COGEM set the prerequisite that "the transgene used does not encode sequences capable of complementing replication-deficient retro- or lentiviral vectors, and does not encode harmful gene products or (proto)oncogenes".[3] In a later advisory report, COGEM specified this prerequisite further, concluding that viral promoter sequences, non-viral introns, internal ribosome entry sites (IRES), and ribosomal skip sequences are not capable of complementing the replication-deficient nature of these vectors, provided that no lentiviral or retroviral sequences are used for this purpose.[4] COGEM later concluded that certain viral promoter sequences would not lead to complementation of a MoMuLV retroviral vector or a SIN lentiviral vector, and that they therefore also fall under the prerequisites set by COGEM.[5]

In theory, recombination with or complementation of the viral vector could also occur in the transduced cells if a related lentivirus or retrovirus is present in the same cell. Regarding retroviral vectors, COGEM has previously stated that the risk of recombination or complementation in patients with acute or chronic HIV and/or HTLV infection is negligible, because MoMuLV has very limited sequence homology and is therefore unable to recombine with or be complemented by human

lentiviruses or endogenous retroviruses. The risk of recombination with, or complementation of, the retroviral vector with gammaretroviruses is also negligible, as there are no known cases of infection of MoMuLV and other gammaretroviruses in humans. In the exceptional case that complementation of the retroviral vector were to occur, this would still pose a negligible environmental risk, as the viral vector is replication-deficient and cannot spread further. Therefore, COGEM advises that HIV- or HTLV-positive patients do not need to be excluded from studies in which retroviral vectors are used.[1]

Originally, one of the prerequisites was to exclude HIV-infected individuals from participating in clinical trials using lentiviral vectors. This condition has since been amended in light of new insights.[3]

For studies in which SIN lentiviral vectors are used, the presence of the SIN deletion in the vectors may reduce, but not entirely prevent, the risk of mobilisation by a possible HIV infection. The risk of mobilisation is further reduced by antiretroviral therapy (ART), which is used to treat HIV infection. These drugs reduce the viral load, which also reduces the risk of transduction by a potentially mobilised vector. Previously, COGEM concluded on theoretical grounds that it is unlikely that recombination between HIV and SIN lentiviral vectors would result in a recombinant virus that replicates better than the wild-type virus. COGEM recently commissioned experimental research into possible recombination between HIV and lentiviral vectors. This research showed that the SIN deletion prevents recombination between HIV and SIN lentiviral vectors.[a] Therefore, COGEM stands by its earlier opinion that HIV-infected individuals may be included in clinical studies with SIN lentiviral vectors.[3]

3. Advice regarding the simplified authorisation procedure

In 2019, the national competent authorities of the EU Member States, in collaboration with the Commission services, drew up a generic 'Good Practice Document' for gene therapy studies involving ex vivo transduced cells.[10,11] Based on this document and COGEM's generic environmental risk assessments,[1,2,3] so-called *permits under fixed conditions* ('vergunningen onder vaste voorwaarden', VoVs) have been drawn up for such studies in the Netherlands, allowing permit applications to be processed via a simplified and shortened procedure.[12,13] These documents set out the requirements that the vectors and GM cells must meet in order to be eligible for a permit under fixed conditions.

As indicated earlier in this advisory report, some of the prerequisites in the COGEM advisory reports on which these VoVs are based have been adjusted in line with new findings. This advisory report outlines the changes that have taken place, and provides a new overview of the prerequisites recommended by COGEM for clinical studies involving ex vivo transduced cells. COGEM recommends that these updated prerequisites be incorporated into the VoVs.

4. Overview of prerequisites for ex vivo retro- and lentiviral transduced cells

COGEM recommends the following prerequisites for the generic environmental risk assessment in clinical studies involving ex vivo retroviral or lentiviral transduced cells:

- The study makes use of retroviral vectors based on the Moloney murine leukaemia virus (MoMuLV), or self-inactivating (SIN) vectors derived from human immunodeficiency virus 1 (HIV-1) and produced using a third-generation production system. These vectors may be pseudotyped with VSV-G or other viral glycoproteins.[14]

a. The report of this research project (*Das AT, Klaver B, Berkhout B. Recombination between HIV and Lentiviral Vectors. An experimental study on recombination between HIV and HIV-derived lentiviral vectors*) will be published shortly.

- The transgene used does not contain sequences which may lead to complementation the replication-deficient retro- or lentiviral vector, or (proto)oncogenes, and does not encode harmful gene products.
    - The use of viral promoter sequences, non-viral introns, internal ribosome entry sites (IRES), and ribosomal skip sequences in the transfer vector is permitted, provided that no lentiviral or retroviral sequences are used.[4]
    - For certain specific lentiviral or retroviral promoter sequences, it has previously been concluded that they will not lead to complementation (see Appendix I).[5] Use of these specific sequences in the transfer vector is permitted.
- For the molecular characterisation, the applicant is required to submit, at a minimum, the vector maps and descriptions of the production plasmids and confirm that these plasmids have been sequenced and that the retro- or lentiviral sequences in the plasmids are identical to the intended sequences.
- The generic environmental risk assessment applies to both autologous and allogeneic ex vivo transduced cells,[b] including macrophages.[14]
- There are a maximum of 500 free infectious vector particles present in the medical product that is given to the patient, corresponding to a reduction ratio of at least 0.002 when using the COGEM formula.[9] This threshold value is recommended regardless of the patient's age and/or blood volume.[14]

If the limit value of 500 free vector particles is exceeded (equivalent to a reduction ratio of 0.002)[9], the following generic measures should be observed in clinical studies with ex vivo **retroviral** or **lentiviral** transduced cells:

- After administration of the medicinal product, the insertion site should be disinfected using an adequate method for inactivating any remaining cells and vector particles;
- Following administration of the medicinal product (ex vivo lentiviral transduced GM cells) the patient should remain in the hospital for at least 16 hours to ensure that the appropriate hospital hygiene measures can be observed. The patient, medical personnel and visitors should be informed about protocols and safety measures concerning the care of wounds and handling of infected material during the first 16 hours after administration of the medicinal product. If the applicant can make a plausible case that the vector particles have already been sufficiently cleared from the patient's body, the advised hospital admission measures can be shortened.

For clinical studies specifically involving cells transduced using **retroviral vectors**, COGEM applies the following additional prerequisites:

- The vector batch, the 'end of production' cells, or the final medical product (the transduced cells) are checked for the absence of replication-competent retrovirus (RCR) using a validated test.

If the above conditions are met, COGEM considers the risks to humans and the environment in clinical studies involving ex vivo retro- and lentiviral transduced cells to be negligible.

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[b] In an advice on allogeneic cells modified to reduce the risk of rejection, COGEM indicated that direct transfer of these cells poses a potential risk to third parties. The authorities involved in the donation of body material must therefore be aware of the possible transfer risks and informed about the patients' treatment, so they can take this into account. COGEM (2020), CGM/201029-01.

5. Updated prerequisites also apply to market authorisation

As COGEM previously advised, the generic environmental risk assessment also applies to market applications for similar GMO products.[3] This means that, when asked to provide advice on market applications for GM medicines or applications that appear to comply with the updated prerequisites of the generic environmental risk assessment, COGEM will issue a shortened opinion referring to the generic advice. By doing so, COGEM hopes to contribute to speeding up authorisation procedures by reducing unnecessary administrative burdens.

Appendix I – List of approved lentiviral and retroviral promoter sequences

Lenti- or retroviral promoter sequences in gammaretroviral vectors

COGEM considers the environmental risk of exchanging gammaretroviral long terminal repeat (LTR) and leader sequences in retroviral vectors to be negligible. This is because no retroviral elements are introduced into the system that could enable recombination and complementation. Therefore, the risk of replication-competent retrovirus (RCR) formation will not increase.

Lenti- or retroviral promoter sequences in lentiviral vectors (3rd generation)

COGEM is of the opinion that using the following lentiviral or gammaretroviral promoter elements to express the transgene in SIN lentiviral vectors produced using a 3rd generation production system poses a negligible environmental risk:

- Murine stem cell virus (MSCV) promoter.
- 'Myeloproliferative sarcoma virus (MPSV) enhancer, negative control region (NCR) deleted, dl587rev primer-binding site (PBS) substituted' (MND) promoter, which is derived from MoMuLV and is also known as MNDU3. This promotor element contains the U3 region of the MoMuLV LTR sequence with the MPSV enhancer, in which the NCR is deleted, and the MoMLV PBS is replaced by the PBS of the MoMuLV strain *dl*587rev.
- Chimeric promoter consisting of a human promoter and a partial LTR sequence of HTLV in which the U3 domain and a part of the U5 domain have been removed.

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