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To the Minister for the Environment and Public Transport
Ministry of Infrastructure and Water Management
Mr Ch. A. Jansen
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DATE 20 May 2025
REFERENCE CGM/250520-01
SUBJECT Amendment of threshold values for free vector particles in gene therapies with GM cells

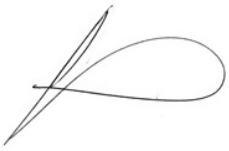
Dear Mr Jansen,

COGEM has reassessed the threshold values for the number of free vector particles in medicinal products used in clinical trials in which patients are treated with ex vivo transduced cells. COGEM therefore notifies you of the following.

Summary:
In certain gene therapies, cells from the patient are genetically altered outside the body, often using retroviral or lentiviral vector particles. Following this procedure, the vector particles are usually removed from the cell by washing before the altered cells are returned to the patient. However, when the cells are returned to the patient there is a possibility that they may still contain some vector particles. The presence of vector particles poses a potential risk if third parties are accidentally exposed to them. In such cases, the possible disruption of cell processes, which could in turn lead to cancer, cannot be ruled out. The number of vector particles that can be unintentionally transferred is therefore a key factor in the environmental risk assessment. The number of vector particles remaining in the cells to be returned to the patient can be determined experimentally or calculated using the 'COGEM formula'. COGEM has previously recommended using a threshold value for the number of vector particles present in the cells as a basis for determining whether or not additional measures are needed to reduce to a minimum the risk of vector particles being transferred. In this advice, COGEM evaluates this threshold value and proposes amending it. COGEM considers that the presence of up to a maximum of 500 retroviral or lentiviral vector particles in the cells to be returned to clinical trial subjects poses a negligible risk to third parties.

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

Yours sincerely,



Professor Sybe Schaap
Chair of COGEM

c.c.

- 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 Water Management
- Dr M.M.C Gielkens, Gene Therapy desk
- Dr K.R.J. Vanmolkot, Central Committee on Research Involving Human Subjects
- K.E. Kok, Ministry of Health, Welfare and Sport
- W.T. Leijs, Ministry of Health, Welfare and Sport

Advise Reassessment of the threshold values for the presence of free lentiviral and retroviral vector particles in clinical trials with ex vivo transduced cells

COGEM Advisory Report CGM/250520-01

1. Introduction

Research on the use of genetically modified (GM) cells in gene therapy trials has increased considerably over the past ten years. In such therapies, cells (usually blood stem cells) are taken from the patient or a donor, genetically modified outside the body (ex vivo), and then returned to the patient. The genetic modification (transduction) is brought about by viral vectors derived from lentiviruses and retroviruses, which insert a therapeutic gene into the genome of the host cell. After transduction, the GM cells often go through various culture and washing steps to remove the excess 'free' (not internalised in the cell) vector particles. However, the possibility that some free vector particles will remain in the GM cells (the medicinal product) to be returned to the patient cannot always be ruled out.

These free vector particles are considered to be a risk factor, because their transmission to third parties (such as relatives) could lead to unintended transduction, which in turn could lead to expression of the transgene in the recipient's cells. Neither can the possibility of healthy cells becoming disrupted be ruled out, which in the most serious scenario could cause cancer.

The number of particles transferred during an unintended exposure is an important factor in the environmental risk assessment.[1,2,3] The number of retroviral and lentiviral vector particles in the medicinal product can be estimated either experimentally or theoretically. In the experimental approach, a validated test can be performed to show that the number of free vector particles has been reduced to a negligible concentration. The number of vector particles present can also be calculated using the 'COGEM formula'.[4,5] When making this calculation, COGEM has recommended using a threshold value[1,6] to determine whether or not additional control measures are needed to reduce to a minimum the risk of free vector particles being transferred from the patient to third parties.

In the meantime, COGEM has advised on a large number of applications for clinical trials with ex vivo transduced cells. In the light of this experience, COGEM decided to re-examine the risks associated with the transfer of free vector particles and reassess the previously recommended maximum admissible number of free vector particles in transduced cells.

2. COGEM advice on clinical trials with retroviral and lentiviral transduced cells

In 2019 COGEM published a generic environmental risk assessment of clinical trials with ex vivo retroviral and lentiviral transduced cells in the absence of free vector particles[1] and in 2020 a generic environmental risk assessment of trials with ex vivo lentiviral transduced cells in the presence of free lentiviral vector particles in the medicinal product.[2] These generic environmental risk assessments are for trials involving the use of retroviral vectors based on the Moloney murine leukaemia virus

(MoMuLV) or the use of self-inactivating (SIN) vectors derived from human immunodeficiency virus 1 (HIV-1) produced using a third generation production system.

The generic environmental risk assessment states various conditions the GMO product should meet to ensure the environmental risk is negligible. These conditions have been revised several times in the light of new understanding.[3,7,8] One of these conditions is the absence of free vector particles in the medicinal product. For clinical trials involving the use of VSV-G (vesicular stomatitis virus G protein) pseudotyped lentiviral vectors, COGEM recommended taking some generic control measures when the presence of lentiviral vector particles cannot be ruled out:

- 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.
- After administration of the medicinal product, the insertion site should be disinfected using an adequate method for inactivating any remaining cells and vector particles, and appropriate hospital hygiene measures should be observed during the care of the patient.
- The patient, medical personnel and visitors should be informed about protocols and safety measures concerning the care of wounds and infected material during the first 16 hours after administration of the medicinal product.

These control measures are based on a worst case scenario in which no reduction of free vector particles has taken place and no more than 10^{12} vector particles are present in the medicinal product.[2] Based on the blood volume of the average patient and the inactivation of vector particles by the complement system (and other processes), it has been calculated that after approximately 12 hours the number of vector particles in the patient's blood will have declined to such an extent that any transfer to third parties will involve a negligible number of vector particles. As a precaution to limit the risk of spread, COGEM advised observing a more generous safety margin and keeping the patient in the hospital for at least 16 hours so that the appropriate hospital hygiene measures can be observed.

2.1 The COGEM formula

The COGEM formula can be used to estimate the number of vector particles still present in the medicinal product (the GM cells). The formula was originally developed for the risk assessment of laboratory work with replication-deficient VSV-G pseudotyped lentiviral vectors (contained use), and for work with lentiviral transduced adherent cells in particular.[4] However, the formula is also used when assessing applications for clinical trials with ex vivo transduced cells.[1,9,10,11] For these applications, the presence (or absence) of both free infectious lentiviral vector particles and retroviral vector particles are calculated.

The COGEM formula calculates the reduction ratio of the number of free vector particles: the reduction in the infectious titre of free vector particles based on a number of inactivating activities. The calculation takes into account the natural inactivation of the vector particles (half-life), inactivation, for example by trypsin, and the loss of particles during washing or passaging of the cell cultures. These reduction steps are related to the total number of infectious particles in the inoculum used for

transduction. In 2020 COGEM commissioned a study to establish an empirical basis for certain assumptions and data were collected on the stability of the lentiviral vector particles.[2,5]

The COGEM formula is:

$$\text{Reduction ratio} = (20^{W} \times 200^{I} \times 2^{FxT})/C_i$$

where **W** is the number of washing steps, **I** is the number of inactivating washes with trypsin and **T** is the culture time in days (24 hours) after transduction. Factor **F** is the reduction factor based on the half-life of the vector particle and is calculated by dividing 24 (the number of hours in a day) by the half-life (in hours) of the vector particle ($T_{1/2}$). This value is heavily dependent on the pseudotyping protein used.[5] $\mathbf{C_i}$ is the original number of infectious vector particles in the inoculum.

The reduction effect of washing steps depends in part on the size of the culture dishes used. For example, for a culture dish with a diameter of 10 cm, the constant value '20' is used, corresponding to a 95% reduction.[5] A constant value of 10 is used as an additional reduction factor for an inactivating washing step with trypsin. Combining the two gives the value in the formula: (10 x 20) = 200. The reduction value of inactivation by trypsin also depends on the pseudotyping protein used.[5]

When the formula gives a reduction ratio of 1, this means that the number of free infectious virus particles has been reduced to 1. A reduction ratio of 100 means that an average of 1/100 = 0.01 infectious particles remain in the cell culture.

2.2 Current threshold values for the number of free vector particles

In clinical trials with ex vivo transduced cells, the cells are introduced into the patient's bloodstream by intravenous infusion. If the medicinal product contains any free infectious vector particles, these could be transmitted to third parties in the event of an incident. The most likely route through which third parties could be exposed to vector particles is via blood-to-blood contact with the patient. The free vector particles transmitted to the recipient could transduce cells, which may then produce the gene product coded for by the vectors. The vector particles may also induce insertional oncogenesis. This is the process by which integration of the vector genome into the DNA of body cells inactivates tumour suppression genes or activates proto-oncogenes, allowing uncontrolled cell growth, or cancer.

To minimise the risk of unintended exposure of third parties to free vector particles, COGEM works with a threshold value for the number of free vector particles in the medicinal product. For gammaretroviral vector particles (based on MoMuLV), this is a reduction ratio of at least 100 (i.e. 1/100 = a maximum of 0.01 vector particles in the medicinal product).[1] For SIN lentiviral vector particles pseudotyped with VSV-G, COGEM recommended a reduction ratio of 1 (i.e. 1/1 = a maximum of 1 vector particles in the medicinal product).[6] If the reduction ratio is not achieved, the control measures previously advised by COGEM should be taken.

The difference in the reduction ratio for these two types of vectors was based on the probability of insertional oncogenesis occurring. In the past this was reported mainly in experiments and clinical trials involving the use of retroviral vectors derived from MoMLV. The probability of insertional

oncogenesis occurring when lentiviral vectors are used was thought to be smaller because of the SIN deletion in these vectors.[2] However, insertional oncogenesis has recently also been observed when SIN lentiviral vectors are used.[12]

3. Reassessment of the threshold values for the number of free vector particles

In 2019 the national competent authorities of the EU member states and the European Commission services jointly drew up a Good Practice document for gene therapy trials with ex vivo transduced cells.[13,14] Based on this Good Practice document and previous environmental risk assessments by COGEM,[1,2,3] standard licensing conditions ('vergunning onder vaste voorwaarden' – VoV) were drawn up for such studies in the Netherlands to simplify and speed up the processing of licence applications.[10,11] This document sets out a number of requirements that the vectors and the GM cells must meet. One of these requirements is the absence of free vector particles in the medicinal product, which are considered to be a risk factor because of the above-mentioned risk of insertional oncogenesis. The Good Practice document refers to the COGEM formula as a tool for a theoretical approach to determining the number of residual vector particles in the medicinal product.

COGEM has advised on a large number of clinical trials. In some of these, use of the COGEM formula indicated a low number of free vector particles in the medicinal product. COGEM points out that while the formula gives the best possible estimate of the number of vector particles, the actual number of vector particles may vary depending on the specific culture and washing conditions. In addition, the formula does not take into account the particles lost by uptake into cells (transduction). COGEM further notes that the presence of small numbers of free vector particles remaining in the medicinal product need not pose a risk. The ultimate risk in the event of such an unlikely exposure will be negligible because the medicinal product, including any remaining free particles, will be diluted after administration into the patient's bloodstream, and the volume of blood that will be involved in such an incident will be very small. In view of the above, the threshold values for the number of free vector particles have been reassessed, taking into account the risk of free vector particles present in the transferred blood volume.

The medicinal product, including any free vector particles that may be present, is administered to the patient through the bloodstream. The most likely route through which a third party could be exposed to vector particles is via blood-to-blood contact. If, after administration of the GM cells to the patient, vector particles are accidentally transmitted from the patient to third parties (such as relatives) via blood-to-blood contact, COGEM has previously estimated that the maximum transmitted volume will be 100 µl.[2] If the previously determined safety margin of a maximum of 0.01 vector particles is applied to this volume and it is also assumed that the average total blood volume of the patient is 5 litres, the suspension with GM cells that is administered to the patient may contain no more than 500 vector particles (corresponding to a reduction ratio of at least 0.002). COGEM no longer considers it necessary to distinguish between gammaretroviral or lentiviral vector particles. The previously advised hospital admission measures (admission for at least 16 hours; see section 2) are only necessary when the medicinal product contains more than 500 vector particles, unless the applicant can make a plausible case that the vector particles have already been sufficiently cleared from the patient's body.

4. Advice

In this advice COGEM has reviewed the environmental risks of the transmission of free retroviral and lentiviral vector particles to third parties in gene therapy trials with GM cells. COGEM considers that at a reduction rate of at least 0.002 (corresponding to a maximum of 500 free vector particles), the likelihood of an adverse effect as a result of accidental transmission is negligible. If this threshold is not exceeded, COGEM considers that the risks associated with the presence of free vector particles in the medicinal product in clinical trials with ex vivo retroviral and lentiviral transduced cells are negligible. If this threshold is exceeded, the previously advised measures involving hospital admission for at least 16 hours should be observed, unless the applicant can make a plausible case that the vector particles have already been sufficiently cleared from the patient's body.

In the Netherlands, licence applications for clinical trials with ex vivo transduced cells can be assessed in a simplified procedure if they meet certain conditions.[10,11] At the moment, a distinction is made between clinical trials involving retroviral or lentiviral vectors in which no free vector particles are present in the medicinal product and clinical trials with SIN lentiviral vectors in which free vector particles may be present in the medicinal product. COGEM advises including the new threshold value in the standard licensing conditions for these trials.

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