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DATE	7 May 2020
REFERENCE	CGM/200507-01
SUBJECT	Generic environmental risk assessment of clinical trials involving free virus particles

Dear Mrs Van Nieuwenhuizen,

Further to the results of a study commissioned by COGEM, which has consequences for the EU environmental risk assessment of gene therapy trials involving ex vivo genetically modified cells, COGEM hereby notifies you of the following.

Summary:
Generic guidance for the environmental risk assessment of gene therapy trials involving the use of human cells that have been genetically modified ex vivo and then returned to the patient has been drawn up for use in the European Union (EU) with the aim of simplifying licence applications for these trials.
The risk assessment contains a number of requirements that must be met. One of these is the absence of 'free' viral vector particles, which may remain as 'residues' after the cells have been genetically modified. The absence of vector particles can be demonstrated experimentally, but can also be estimated theoretically by calculation. The EU risk assessment refers to a formula developed earlier by COGEM for this purpose.
COGEM has now had this formula experimentally validated for the most commonly used viral vectors, the lentiviral vectors. The study shows that the formula needs to be adjusted and that in the past the number of free vector particles has been underestimated. As a consequence, when assessing future licence applications it will probably not always be possible to ascertain with certainty that medicinal products administered to a patient no longer contain vector particles, in which case the application would not meet the requirements of the EU environmental risk assessment.
Based on a theoretical worst case scenario, COGEM calculated the likelihood of third parties becoming exposed to free vector particles during such trials. Given that the viral particles appeared to be cleared from the patient within 16 hours after the medicinal product was administered and transmission can be prevented by observing standard hospital hygiene measures and several additional measures, COGEM advises that patients should remain in the hospital for at least 16 hours after being given the medicinal product. If these conditions are taken into account, COGEM is of the opinion that trials with genetically modified cells in which free lentiviral vector particles are present can meet the requirements for assessment under the EU generic environmental risk assessment.

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

Yours sincerely,

Professor Sybe Schaap
Chair of COGEM

c.c.
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Generic environmental risk assessment of clinical trials involving ex vivo lentiviral transduced cells: Presence of free vector particles in the medicinal product

COGEM advice CGM/200507-01

1. Introduction

An increasing number of gene therapy trials involve the use of genetically modified (GM) cells. In these trials, cells are taken from the patient, genetically modified and then returned to the patient. The genetic modification (transduction) is effected by viral vectors derived from lentiviruses and retroviruses (lentiviral and retroviral vectors; see §2). After transduction, the GM cells go through various culture and washing steps to remove the excess 'free' (not internalised in the cell) vector particles.

These gene therapies have proved to be highly successful, including for the treatment of certain types of blood cancers, such as leukaemia.[1,2,3] In response to the strong growth in the use of these types of gene therapies, in 2019 the national competent authorities of the EU Member States and the Commission services jointly drew up a Good Practice document with the aim of facilitating and streamlining the environmental risk assessment of licence applications for these trials.[4,5] The Good Practice document provides guidance specifically on the environmental risk aspects of the use of lentiviral and retroviral vectors and sets down 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 GM cell suspension (the medicinal product). These free vector particles are considered to be a risk factor because they could cause the growth of tumours (see §2.2 and §2.3).

The absence of free lentiviral or retroviral vector particles can be experimentally determined by means of a validated test, for example by analysing the culture supernatant or washing liquid during culture or washing of the GM cells to measure the reduction in the number of free vector particles.[6]

It is also possible to theoretically estimate the reduction in the number of free vector particles by using the formula previously developed by COGEM (the 'COGEM formula').[7] This formula is used to estimate the reduction in the number of free vector particles during the preparation of the GM cells in relation to the number of vector particles added during transduction, the result being expressed as a reduction ratio. It is assumed that a reduction ration equal to or greater than the minimum threshold value is an indication that no free vector particles remain in the medicinal product.

The EU Good Practice document refers to the COGEM formula and sets a minimum reduction ratio of 100 for lentiviral and retroviral vector particles,[5] which is equivalent to a maximum of 0.01 free vector particles per batch of GM cells.

In 2019 COGEM published an advice on a generic environmental risk assessment of gene therapy trials involving the use of ex vivo GM cells.[7] In its advice, COGEM raised the minimum threshold value for the reduction ratio for certain lentiviral vectors to 1 (equivalent to a maximum of one vector particle per

batch of GM cells), because the risks to human health and the environment of using this type of vector are considered to be lower.

The advice also mentions that COGEM was waiting for the results of an ongoing study into the stability of lentiviral vector particles. The aim of the study was to experimentally substantiate a number of assumptions in the COGEM formula and obtain new data on the stability of lentiviral vector particles. The study has now been completed and the implications of the relevant findings (see §3) have been incorporated into this present generic advice.

1.1 Research into the COGEM formula and implications of the results

The COGEM formula contains a number of parameters. The values of these parameters are based on limited data from the literature and several theoretical assumptions.[8,9] The study showed that most of the parameters in the formula need to be adjusted because the values obtained experimentally were different than had previously been assumed. Calculations made in the past using the original values have been shown to underestimate the number of free particles (for a more detailed explanation, see §3.2).

Based on its experiences with previously assessed gene therapy trials, COGEM estimates that when assessing future licence applications it will not always be possible to rule out that the number of free vector particles has not been reduced sufficiently to meet the minimum threshold in the EU Good Practice document. This means that when the medicinal product is administered to the patient the GM cell suspension may still contain infectious vector particles.

1.2 Purpose of COGEM's advice

COGEM proposes the use of general control measures for gene therapy medicinal products containing residual free 'self-inactivating' (SIN) VSV-G pseudotyped lentiviral vector particles. If this advice is observed, COGEM is of the opinion that the risks to human health and the environment of these trials will be negligibly small. In this sense, this advice is supplementary to COGEM's previous advice on the generic environmental risk assessment of clinical trials involving ex vivo retroviral and lentiviral transduced cells.[7] Current advice first briefly discusses the main differences between lentiviral and retroviral vectors and explains the possible risks of the presence of residual free vector particles in gene therapy medicinal products containing ex vivo transduced cells (see §2). The findings of the study are then briefly explained (see §3). The final section sets out the reasoning behind the control measures recommended by COGEM (see §4).

2. Background information on lentiviral and retroviral vectors

Lentiviral and retroviral vectors are used in many gene therapies because the genetic material of these vectors integrates into the genome of the host cells. Further information on the viruses from which these vectors are derived is given in the text box 'Lentiviruses and retroviruses'.

Lentiviruses and retroviruses

Lentiviruses and retroviruses are two genera within the Retroviridae family.[13] The viruses have a single-stranded RNA genome which is converted by a virus-specific polymerase – reverse transcriptase – into a double-stranded (ds) complementary DNA, which integrates into the genome of the infected cell.[14,15] In recent years, gene therapies have made increasing use of vectors derived from mouse gammaretroviruses or vectors derived from human lentiviruses.

The RNA genome of lentiviral and retroviral particles is enclosed within capsid proteins and surrounded by a lipid membrane, a structure called the envelope. The viral particles contain all the components needed to initiate the viral life cycle, including the reverse transcriptase, integrase and protease enzymes. These proteins are encoded in the viral genome by the *pol* gene. Embedded in the lipid membrane are the envelope proteins, which determine the host tropism of the virus. These proteins are encoded by the *env* gene.

The life cycles of lentiviruses and retroviruses are similar. Infection of the host cell is induced by specific binding of the envelope proteins onto a cellular receptor, sometimes in combination with a co-receptor. During this process the viral particle is partially dismantled. In the cytoplasm the viral RNA is synthesised through a complex series of steps into a ds DNA copy, which integrates into the cellular genome. Identical long terminal repeats (LTRs) are included at both the 5' and 3' ends of the proviral DNA. These sequences play a key regulatory role in the integration of the proviral DNA and the transcription of the viral genome.

The transcription machinery of the host cell synthesises the viral RNA from the integrated proviral DNA. After transport to the cytoplasm, two copies of the viral RNA are enclosed within the capsid protein (encoded by the *gag* gene). Essential for the binding of the capsid protein with the viral RNA and the eventual formation of the viral particles is the packaging signal Ψ in the RNA. The infectious viral particles are formed during the budding on the plasma membrane, a process in which the enclosed viral genome is surrounded by a lipid membrane containing integrated envelope proteins.

An important difference between retroviruses and lentiviruses is that lentivirus ds DNA is actively transported into the cell nucleus via the nuclear pore complex, while retroviruses must wait until the nuclear membrane has disassembled during mitosis. As a consequence of this, retroviruses and the vectors derived from them, unlike lentiviruses, can only infect dividing cells. Another important difference between retroviruses and lentiviruses is that in addition to the previously mentioned genes *pol*, *env*, and *gag*, the lentiviruses contain the four accessory genes *vif*, *vpr*, *vpu* and *nef* and the two regulatory genes *rev* and *tat*.

2.1 Risks of free vector particles in GM cell suspensions for gene therapy applications

The presence of residual free vector particles in GM cell suspensions (the medicinal product) is considered to be a risk factor. After the product has been administered to the patient, the vector particles can transduce cells (secondary transduction). Subsequently, the sequence encoding the vector genome

can integrate into the cellular genome. This process is called insertional mutagenesis.[a] In the worst case, insertional mutagenesis can cause cancer by inactivating a tumour suppressor gene or activating a proto-oncogene (oncogenesis).[10,11] As secondary effects, the infectious vector particles can also trigger an immune response, or the secondary transduction can express the transgene in cells other than the target cells.[10]

2.1.1 Risks of gammaretroviral vectors

COGEM notes that the occurrence of insertional oncogenesis is based primarily on data from experiments and clinical trials with GM cells ex vivo transduced with gammaretroviral vectors derived from the Maloney murine leukaemia virus (MoMLV). These vectors have a preference for integration near transcription start sites, where promoter activity at the ends of the inserted vector genome (the 3' long terminal repeat (3' LTR)) can lead to activation of proto-oncogenes present in the host genome.[12,13,14] To date, three clinical applications in which cells were transduced ex vivo with MoMLV-based vectors have been reported as having led to leukaemia caused by the activation of a proto-oncogene or inactivation of a tumour suppressor gene as a result of the insertion of the transgene.[15,16,17,18,19] This occurred in the first clinical applications using this type of vector: gene therapies for the treatment of X-linked Severe Combined Immunodeficiency (X-SCID),[20,21] X-linked Chronic Granulomatous Disease (X-CGD)[17] and Wisskott-Aldrich syndrome.[22] In total, 14 of the 32 treated patients were affected.

It should be noted that no insertional oncogenesis was observed in a clinical trial of a treatment for Adenosine Deaminase (ADA-)SCID (42 patients), even though the same MoMLV backbone was used for the transduction vector.[23,24,25] A study of the integration sites in the genome in the X-SCID and ADA-SCID trials showed little difference.[26,27,28] It is possible that other factors also play a role in triggering oncogenesis, such as the transgene that is used.[29]

2.1.2 Risks of lentiviral vectors

Other vectors that are frequently used for ex vivo mediated transduction of cells are third generation SIN lentiviral vectors. These vectors are derived from the *Human immunodeficiency virus 1* (HIV-1) and they lack a specific domain in the 3' LTR, which considerably reduces the chance of mobilisation of the vector from the host genome. In addition, it is assumed that activation of proto-oncogenes following integration of the vector DNA is not possible.[30]

Since the first application of these lentiviral vectors in 2009, no reports have been made of tumour formation as a result of insertional mutagenesis that can be attributed to use of the vector.[31,32,33,34,35,36] In recent years many clinical trials have been conducted with T cells and CD34+ haematopoietic stem cells that have been ex vivo transduced with this type of lentiviral vector.[37] As far as is known, no cases of leukaemia have been reported. However, benign clonal expansions of cells were reported in a gene

[a] COGEM notes that the terms insertional mutagenesis and insertional oncogenensis are sometimes used interchangeably. Where the integration of the vector genome into the cell genome leads to the development of cancer, COGEM uses the term insertional oncogenesis. Where the integration does not lead to cancer, the term insertional mutagenesis is used.

therapy trial of a treatment for adrenoleukodystrophy[31] and in a gene therapy trial of a treatment for thalassemia.[38] This indicates that insertion of the vector genome can lead to unanticipated effects.

It is reported in the literature that when SIN lentiviral vectors are used, after the transgene has integrated into the genome it is possible that read-through transcription of the transgene may occur, which can lead to the formation of unintended and undesirable viral-cellular fusion transcripts.[39] Consequently, research is still being conducted into lentiviral vectors to further improve their biosafety and improvements are still being made to these vectors.[40]

3. Study to experimentally validate the COGEM formula

The study to experimentally validate the COGEM formula was carried out by Dr I.J.C Dautzenberg and Professor R. C. Hoeben of Leiden University Medical Center (LUMC). The resulting research report 'The COGEM formula revisited. Experimental validation of the reduction ratio formula for free lentiviral particles' (CGM 2020-01)[41] can be downloaded from the COGEM website.

The aim of the study was to experimentally validate the values used for the parameters in the COGEM formula for third generation SIN VSV-G pseudotyped lentiviral vector particles and to investigate if these validated values are equally applicable for this type of vector particle when pseudotyped with other heterologous envelope proteins. These alternative envelope proteins are from feline endogenous virus RD114, *Gibbon ape leukaemia virus* (GALV), MoMLV (both ecotrope and amphotrope), the hemagglutinin (H) and fusion (F) glycoproteins of *Measles morbillivirus* (measles virus) and the glycoprotein of *Rabies lyssavirus* (rabies virus).

COGEM notes that VSV-G is used in many clinical trials and laboratory experiments. The other envelop proteins are mainly used in laboratory experiments.

3.1 The COGEM formula explained

The COGEM formula was originally developed to assist the risk assessment of laboratory applications involving replication-deficient VSV-G pseudotyped lentiviral vectors (contained use), especially for applications with lentiviral transduced adherent cells.[8,9] The formula is used to calculate the reduction ratio, as described above. This ratio represents the reduction in the infectious titre of free replication-deficient vector particles following preparation of the GM cells.

Factors that contribute to the reduction in infectious titre are (a) the 'natural' inactivation of the infectious particles (based on the halftime), (b) the inactivation of particles under the influence of trypsin or human serum, (c) the loss of particles during washing or passaging of the cell cultures, and (d) the loss of particles due to cellular uptake (transduction). The combined effects of these reduction steps can be related to the total number of infectious particles in the inoculum used for transduction (e).

Factors a, b, c and e are included in the COGEM formula. Factor d is not taken into consideration because it is hard to standardise and varies per experiment. The factors are included in the formula as follows:

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

where W is the number of washing steps, I is the number of inactivating washes with trypsin or human serum and T is the culture time in days (24 hours) after the start of transduction. Factor F is calculated by dividing 24 (the number of hours in a day) by the halftime (in hours) of the vector particle ($T_{1/2}$). Ci is the original number of infectious vector particles in the inoculum. The constant 20 (corresponding to a 95% reduction) was determined by expert judgement.[8,9] The value of $T_{1/2}$ for VSV-G pseudotyped lentiviral vector particles is taken to be 10. The value 10 is also assumed when calculating the additional reduction factor for an inactivating washing step with trypsin or human serum (10 x 20 = 200). Both values ($T_{1/2}$ and the additional reduction factor) were derived from the sparse data available in the scientific literature at the time.[42,43,44] For retroviral vectors based on MoMLV, various values for $T_{1/2}$ at 37ºC are given in the literature, depending on the pseudotyping, ranging from 4 to 13 hours.[44,45,46,47,48,49]

3.2 Research results

The results indicate that the basic structure of the formula is sound, but that most of the values of the parameters have to be adjusted,[41] leading to a lower reduction ratio in practice than was previously assumed. For example, the experiments show that the stability of the vector particles (the natural decay) is heavily influenced by the culture conditions under which transduction took place and that the stability (reflected in the form of $T_{1/2}$ and the reduction factor F derived from it) is heavily dependent on the pseudotyping (see p. 32–33 of the report). In practice, trypsin is less effective at inactivating the vector particles than was assumed, and the degree of inactivation is also heavily dependent on the pseudotyping (see p. 30–31 of the report). The researchers also conclude on the basis of data from the literature that the degree of complement-mediated inactivation depends on the serum donor (see p. 38–39 of the report). Finally, the researchers note that the titre for a vector batch depends on the type of assay used (see p. 34–36 and 40–41 of the report). This means that certain titre calculations can lead to an underestimation of the initial inoculum. The researchers therefore propose multiplying the value for the initial inoculum by 10.

A ‘revisited COGEM formula’ is presented on page 46 of the report, along with a table of values for the F factor and the reduction factor after trypsin inactivation (Tryp) for each pseudotyping. A simplified form of this table is given below.

The authors emphasise that the revisited formula should only be used for cultures of adherent cells, that the reduction effect of washing steps depends on the size of the culture dishes used and that the values apply only to lentiviral vectors.

TABEL Reduction factors and halftimes (p. 31, 33 and 46 of the research report)

	Tryp[1]	**$T_{1/2}$ (in hours)**	**F**
VSV-G	1	34.7	0.7
Measles	4	14.1	1.7
GALV	79	8.3	2.9
Rabies	2	15.4	1.6
RD114	110	21.2	1.1
MoMLV 4070A (amphotrope)	4	36.6	0.7
MoMLV 10A1 (amphotrope)	-	13.8	1.7
MoMLV (ecotrope)	10	18.6	1.3

[1] Trypsin treatment of cells (at least 5 minutes at 37°C)

4. Considerations

COGEM notes that in various trials involving GM cells transduced with VSV-G pseudotyped lentiviral vectors, it has been demonstrated both in vitro and in vivo that not all vector particles were removed from GM cell suspensions following culture and washing procedures.[50,51,52,53,54] During clinical trials, therefore, not only the patient, but also third parties could be exposed to free vector particles in the medicinal product. COGEM therefore considers it important that the environmental risk assessment addresses the question of whether or not such an exposure could occur, and if so, to what degree.

4.1 Ways in which third parties can be exposed to free vector particles

In clinical trials involving ex vivo transduced cells, these cells are introduced to the patient's bloodstream by intravenous infusion. COGEM notes that publications on these gene therapy trials contain little or no data indicating whether or not infectious vector particles are shed, for example via urine, faeces or other bodily secretions. Such shedding could cause third parties to be exposed to the vector particles.

Studies in mice administered VSV-G pseudotyped lentiviral vector particles intravenously have shown that the vector particles were not shed via faeces or urine and no germline transmission took place.[55,56] Neither was there any aerogen transmission when treated and non-treated mice ('sentinel animals') were kept in the same cage.[56]

According to scientific publications, the most likely routes through which third parties could be exposed to vector particles are blood to blood contact (such as needlestick incidents) and contact transmission (for example, touching mucous membranes of the eye, nose or mouth with a contaminated hand).[57]

COGEM is of the opinion that the above-mentioned experimental 'shedding data' from mice in which it has been demonstrated that no shedding occurred via bodily secretions can be extrapolated to humans.

This also takes account of the fact that before the vector particles end up in urine, faeces or other bodily secretions they will have had to pass through many types of cells, which considerably increases the likelihood of transduction. The number of free particles will be reduced as a result. In addition, there are no reasons to assume that any residual vector particles will remain stable under the environmental conditions prevailing in urine and faeces.

Taking the above into consideration, COGEM concludes that the most likely routes through which third parties could be exposed to vector particles are blood to blood contact and contact transmission from contaminated hands (with blood) to mucous membranes in the mouth, nose or eyes, and estimates that the risk of transmission is greatest from blood to blood contact. COGEM considers the likelihood of third party exposure to vector particles via aerogen transmission or shedding via urine or faeces to be negligibly small.

4.2 Decrease in the presence of vector particles in the patient

In the clinical trials that COGEM has evaluated in which ex vivo transduced cells were used (17 trials carried out in the period from 2011 to the beginning of 2020) the number of infectious vector particles used for the transductions varied from 10^6 to 10^{12} particles.[58,59] Assuming a theoretical worst case scenario, this means that the medicinal product could contain up to 10^{12} particles. Based on a typical adult blood volume of approximately 5 litres, the concentration of vector particles in the patient's blood after being given the medicinal product will be about 2×10^8 particles per ml ($10^{12}/5000$).

COGEM notes that this highly theoretical scenario is an overestimate and ignores the fact that the number of free vector particles in the medicinal product will decrease during the preparation of the GM cells as a result of various factors, including biological decay and the washing steps.

If after administration of the medicinal product an incident occurs in which vector particles are transmitted to a third party (for example through blood to blood contact or contamination of mucous membranes via contact with hands contaminated during care of the insertion site), COGEM estimates that the maximum volume that can be transmitted is 100 µl. Assuming a vector particle concentration of 2×10^8 particles per ml (see above), this means that in the worst case scenario the maximum amount transmitted will be 2×10^7 vector particles.

The free vector particles still present in the GM cell suspension will decrease in number after administration to the patient through natural decay, inactivation by exposure to complement and possible secondary transduction by body cells. DePolo *et al*. (2000) and Schauber-Plewa *et al*. (2005) have demonstrated in vitro that VSV-G pseudotyped lentiviral vector particles produced on human cells can be inactivated by human serum at 37 ºC, but that the inactivation differs widely from donor to donor.[43,60] Schauber-Plewa *et al*. have also demonstrated that the serum inactivation differs considerably depending on the pseudotyping. For example, amphotrope pseudotyped lentiviral vector particles were shown not to be inactivated by human serum.[60]

The inactivation of the vector particles is mediated by the complement system in the blood (a component of the innate (non-specific) immune system) and antibodies also appear to play a role.[43,60,61]

Comparison between donors shows that the reduction in the number of vector particles after an hour ranged from a factor of 6 to a factor of 100.[60] In rats is has been shown that one hour after intravenous administration of 10^7 VSV-G pseudotyped lentiviral vector particles the amount still remaining in the blood plasma was found to be less than 1%.[62]

Following a transmission incident involving 2 x 10^7 vector particles, complement inactivation and other factors will, in the least favourable case (a reduction factor of 6 per hour), after one hour have reduced the number of vector particles in the bloodstream to ⅙ x 2 x 10^7 = 3.3 x 10^6. After 9.5 hours there will be less than one vector particle left ((⅙)$^{9.5}$ x 2 x 10^7 = 0.8 particles). The threshold value in the EU Good Practice document of 0.01 vector particles (see §1) will be reached after 12 hours.

Taking this highly unlikely worst case scenario into account, COGEM concludes on the basis of the above reasoning that the number of vector particles in the bloodstream of the patient after about 12 hours will have been reduced to a negligible number.

COGEM notes that this scenario is based on an extrapolation of experimental data involving several assumptions. An important assumption is that the inactivation in the bloodstream occurs in the same way as in serum in vitro. However, COGEM does not rule out the possibility that this kinetics is different in vivo, for example because the vector particles break down more slowly or more quickly in the body, or because the particles bind to body cells, leading to a decrease in number due to secondary transduction. Another important assumption is the previously mentioned fact that the theoretical scenario ignores the reduction in free vector particles during the preparation of the medicinal product.[b]

Taking all the above into account, COGEM considers it advisable for precautionary reasons to apply a generous safety margin to ensure the vector particles are cleared from the body of the patient and to allow at least 16 hours for this to occur (instead of the theoretically calculated 12 hours).[c]

4.3 Control measures

As previously mentioned, COGEM considers that the transmission of vector particles to third parties is most likely during transfusion or contact with the patient's blood. A measure to minimise this risk is to keep the patient in the hospital for at least 16 hours after the medicinal product is administered (see §4.2). Other control measures include adequate disinfection of wounds to inactivate any vector particles

[b] COGEM points out that the findings of the underlying research report[41] and other sources indicate that for the environmental risk assessment it is desirable that more research is undertaken into the development of securely validated assays to determine the titre of infectious particles in vector batches and into securely validated methods for inactivating free infectious vector particles in GM cell suspensions. It is also desirable that the data obtained from the use of these methods are made available.

[c] COGEM notes that so far the medical protocols for these types of gene therapy trials require that treated patients remain admitted in the hospital for 7 days so that any resulting adverse effects arising in the patient, such as cytokine release syndrome, can be treated.

that may be present in the blood and observing standard hospital hygiene measures during the care of the patient (as set down, for example, in the gene therapy guidelines drawn up by the Infection Prevention Working Group (WIP)).[63,64] COGEM notes that the risk of exposure to vector particles is greatest at or in the vicinity of the infusion site shortly after the administration of the medicinal product.

Finally, COGEM considers it important that the patient and their relatives and close friends (for example present during visiting hours) are informed about the nature of the medicinal product and the possible risks of exposure during the first 16 hours after the medicinal product is administered to prevent them coming into contact with potentially contaminated materials or blood.

5. Conclusions and advice

The outcome of the experimental validation of the COGEM formula is that the values of the parameters that have been used in the equation need to be adjusted.[41] COGEM concludes that the research results imply that it is possible that not all gene therapy applications in which ex vivo transduced cells are used will be able to meet the required threshold value in the EU Good Practice document for the reduction ratio of vector particles. From its previous experiences with licence applications, COGEM acknowledges the possibility that in future more licence applications will be submitted for treatments in which the medicinal product to be administered to the patient (the GM cell suspension) may contain residual amounts of non-internalised infectious lentiviral vector particles. Although highly unlikely, based on our current understanding it cannot be ruled out that exposure to these particles may lead to adverse effects, including insertional oncogenesis (see §2.2 and §2.3).

COGEM therefore considers it important that to ensure human and environmental safety, the likelihood of third parties being exposed to 'free' vector particles during this type of clinical trial is kept to the minimum. Based on a theoretical calculation (see §4.2) it concludes that in the most unfavourable case, and observing a generous safety margin, the number of vector particles remaining in the patient 16 hours after the medicinal product is administered will have decreased to a negligible amount. COGEM is therefore of the opinion that, if the GM cells used in the gene therapy are transduced with SIN replication-deficient VSV-G pseudotyped lentiviral vectors and the correct measures are taken, the risks to human health and the environment are negligibly small, irrespective of the inserted transgene.

Should the medicinal product still contain free infectious lentiviral vector particles, COGEM recommends that, supplementary to its previous advice,[7] the following additional generic measures should be taken into account:

- 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 standard hospital hygiene measures can be observed.
- After administration of the medicinal product, the insertion site should be covered and the WIP guidelines for gene therapy observed.

- The patient, medical personnel and visitors should be informed about protocols and safety measures concerning care of wounds and contaminated material during the first 16 hours after administration of the medicinal product.

If these additional conditions are taken into account, COGEM is of the opinion that the risks to human health and the environment of gene therapy trials involving GM cells that have been ex vivo transduced with a lentiviral vector (SIN, VSV-G pseudotyped) – and in which at the time of administration it cannot be ruled out that the medicinal product still contains free lentiviral vector particles – are negligibly small.

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