Rijksinstituut voor Volksgezondheid
en Milieu
*Ministerie van Volksgezondheid,
Welzijn en Sport*

Aanvullende informatie formulier:
versie 1.02

AANVULLENDE INFORMATIE:

- U dient dit formulier uitsluitend te gebruiken voor het aanleveren van aanvullende informatie op verzoek van Bureau GGO.
- Eventuele vertrouwelijke informatie dient in afzonderlijke bijlages meegezonden te worden.
- De afhandeling van de kennisgeving of de vergunningverlening is een openbare procedure en daarmee zijn alle in de kennisgeving of aanvraag verstrekte gegevens openbaar. Informatie over het vertrouwelijk houden van gegevens en op welke wijze dit aangevraagd moet worden vindt u op de website www.ggo-vergunningverlening.nl.
- Dit formulier hoeft niet ondertekend te worden en kan eventueel ook per mail worden aangeleverd.
- Een algemene technische toelichting voor het gebruik van dit formulier is ook te vinden op de website www.ggo-vergunningverlening.nl.
- Contactgegevens:
  - Internet: www.ggo-vergunningverlening.nl
  - Email: bggo@rivm.nl
  - Telefoon: 030-274 2477

Aanvullende informatie formulier

Dit formulier kan gebruikt worden om aanvullende informatie aan te leveren op verzoek van Bureau GGO.

Subdossiernummer

Met subdossiernummer wordt een nummer in het volgende format bedoeld: IG 15-123_IIk-000 of IG 15-456_III-003.

subdossiernummer: IM-MV 16-006

Verzoek tot aanvullende informatie

Op welke wijze is het verzoek tot aanvullende informatie ontvangen:

- (●) brief
- ( ) e-mail

Geef het kenmerk van de brief van Bureau GGO voor verzoek tot aanvullende informatie:

brief kenmerk: IM-MV 16-006_000.vr.4

Aanvullende informatie

antwoord op de door Bureau GGO verzochte informatie:

RE: IM-MV 16-006 responses to GMO Questions on KTE-C19

Dear GMO Office Board of Management,

In response to your comments IM-MV 16-006_000.vr.4 dated 28 July 2016 and the email received August 10th 2016 we made changes to the application form.
The Questions are repeated in the order received and the answers are provided below each respective question for ease of review.

The changes made to the application form are in red.

Question 1
In addition to an application form showing the changes, you should also submit a clean document with the application.

Re Question 1: *Both a 'track changes' and an 'accept changes' version of the revised (sept. 2016, revision **2**) application are now submitted. For your records, also a clean version of our previous submission (july 2016, revision **1**) is enclosed.*

Question 2
You state that sampling and sample preparation is not part of this application for a permit (inter alia A1.4, A1.10, A4.7). You should be aware that if sampling is not part of the permit, you cannot take samples from treated patients (as stated in A4.6 and A4.8). There are also contradictory aspects in your current application. You specify (inter alia A1.4, A4.7) that sampling does not take place and is not part of the application. However, in A4.6, A4.7 and A4.8 you state that sampling does take place and that patients' blood will be tested for the presence of RCR. Please clarify this and make your application consistent.N.B.: If sampling is part of the permit, you can present well-founded arguments to explain why no additional safety measures are taken during sampling and subsequently during storage, transport and processing of the samples. These samples can, if desired, be processed under a separate restricted use permit.

Re Question 2: *We reinserted the section that was in the original application concerning sampling of the patients and would like to re-incorporate sampling as part of the application. This information is now present in A1.4, A1.10 and A4.7. As corroborated in sections A3.2 and A4.6, samples taken from the patient will not contain RCR and therefore do not fall under `ingeperkt gebruik'/contained use measures and no additional safety measures (on top of the regular hospital measures dealing with human diagnostic samples) are necessary. Samples can therefore be taken at any location either at the hospital, at the outpatient department or even in another hospital should that be necessary for whatever reason. The reason the sponsor will test samples for RCR (at a central laboratory) is to extend the current database of samples tested for RCR as an extra safety measure*.

Question 3

You state that the entire DNA sequence of pMSGV-FMC63-CAR has been confirmed by DNA sequencing. In that case, what is

your reference standard for the pMSGV vector?

Re Question 3: *The construction and description of the reference standard vector from which the reference standard sequence is derived is described in detail in Kochenderfer et al. 2009. Briefly, the anti-CD19 scFv was inserted into the MSGV (mouse stem cell virus-based splice-gag vector)-CD28-human TCR-ζ retroviral backbone. This parental plasmid is the reference sequence for all subsequent sequence verification studies. This information is present in A2.12.*

Question 4

In addition to the 3' LTR of MoMLV, is there also a heterologous polyadenylation (polyA) sequence present at the 3' end of the transgene (FMC63-CD28-CD3zeta)? If so, what is the origin and the exact position of this polyA sequence?

Re Question 4: *There is no heterologous polyadenylation signal in the pMSGV1 plasmid containing the FMC63-CD28-CD3zeta CAR. This vector uses the native polyadenylation signal within the LTR. This information has been added to A2.8.*

Question 5

In A2.8 it is stated that 'the original envelope from the retrovirus has been removed and integrated into the PG13 cell line.' You should confirm that 'and integrated into the PG13 cell line' can be deleted and that only the GALV envelope is present in the PG13 cell line.

Re Question 5: *The text in A2.8 has been clarified as follows:*

In the modified vector, the original envelope from the retrovirus has been removed. All the other components from the retrovirus have been integrated into the PG13 cell line, including the genes gag, pro and pol. A different envelope derived from Gibbon ape leukemia virus (GaLV) has also been integrated in the PG13 cell line in order to modify the tropism of a retroviral vector produced in these cells.

Question 6

In A2.10 you state that Phoenix Eco cells are transfected with pMSGV-1-TCR or - CAR. You should confirm that you are only going to perform transfections with CAR, as stated in A2.11 and 2.14.

Re Question 6: *For the purpose of this application, Kite Pharma will only perform transfections with CARs. The text in A2.10 has been updated as follows:*

"Phoenix ECO cells were transfected with plasmid DNA (pMSGV1-FMC63z-CD28z, see Figure 1)"

Question 7

In A2.12 you state that the DNA sequence of the CAR-coding region and the flanking sequences have been confirmed in *'cells at the limit of in vitro cell age from a GMP production batch'* of the PG13-CD19-H3 vector. You should explain this in more detail as it is unclear what is meant by this. You should also clarify what is meant by 'flanking sequences'? Is this a reference to the flanking sequences of the CAR in the vector pMSGV1_FCM63_CD28z (i.e. psi and the parts derived from CD28 and CD3) or to the flanking regions outside the 5'LTR and 3'LTR in the MCB? How large is this flanking region?

Re Question 7: *For clarification A2.12 has been re-written as follows:*

The complete DNA sequence of plasmid pMSGV-FMC63-CAR was confirmed by DNA sequencing. After construction of the cell line used to produce PG13-CD19-H3 Vector, the DNA sequence of the proviral DNA was confirmed from the Master Cell Bank (MCB) (PG13-CD19-CAR-H3 MCB) and compared to the sequence of plasmid pMSGV1-FMC63 CAR. To do this, genomic DNA was isolated from a single PG13-CD19-CAR-H3 MCB vial. Using high-fidelity PCR, proviral DNA from the MCB was amplified and sequenced to ensure sequence integrity of the integrated transgene. The region of the proviral DNA encoding the CAR sequence was sequenced and confirmed as a 100% sequence match to the parent control plasmid MSGV-FMC63 CAR used to create the MCB. The construction and description of the reference standard plasmid is described in detail in Kochenderfer et al. 2009. Briefly, the anti-CD19 scFv was inserted into the MSGV (mouse stem cell virus-based splice-gag vector)-CD28-

human TCR-ζ retroviral backbone to create the final retroviral transfer vector.

Additionally, the DNA sequence of the CAR coding region was confirmed in cells expanded from a cell bank vial at the end of a GMP production batch of PG13-CD19-H3 Vector (cells at the limit of in vitro cell age, as outlined in ICH Harmonised Tripartite Guideline, Viral Safety Evaluation Of Biotechnology Products Derived From Cell Lines Of Human Or Animal Origin, Q5A(R1), 1999).

The DNA sequence of the CAR gene aligned 100% with the expected sequence in of the control plasmid MSGV1-FMC63-CAR used to create the PG13-CD19-CAR-H3 MCB. In addition, the DNA sequence both upstream (100 base pairs within the CAR coding region to 823 base pairs upstream) and downstream of the CAR gene coding region (97 base pairs within the coding region to 316 base pairs downstream of the coding region), were also confirmed as identical to the MSGV1-FM63-CAR reference sequence by DNA sequencing of PCR amplified genomic DNA. The DNA sequence of the CAR coding region is not confirmed in patient cells for each lot of KTE-C19. The assessment of a functional CAR protein in each batch is assessed by demonstrating specific potency, specifically by measuring IFN-gamma production after co-cultivation with antigen-positive target cells.

Question 8

In A2.13 you describe the production of the PG13 packaging cell line. This shows that pLN has also been introduced into the PG13 cells. This sector contains a packaging signal in addition to two LTRs. Has pLN actually been introduced into the PG13 cells? Do the PG13 cells continue to produce viral particles that contain the neomycin resistance gene? You should clarify this and include all possible consequences of it in your environmental risk analysis.

Re Question 8: *A2.13 has been updated as follows:*

"PG13 cell line was derived from TK-NIH/3T3 cells (murine fibroblast), a spontaneously immortalized cell line originally isolated from Swiss albino mouse embryo tissue and deficient for Thymidine Kinase. The MoMLV gag-pro-pol was introduced using the pLGPS plasmid and the GaLV envelope using pMOV-GalV. To test the feasibility of this approach to produce recombinant retrovirus the transfer plasmid pLN, containing a neomycin resistance gene, was used in the original study to test retroviral production (Miller et al., 1991; Miller and Rosman, 1989). Subsequently, pLN was not used during generation of the expression cell line for production of PG13-CD19-H3 Vector. The parental PG13 packaging cell line only contained integrated pLGPS and pMOV-GaLV plasmids depicted in Figure 3. The parental PG13 cell line does not contain integrated neomycin resistance gene and does not produce retrovirus containing the neomycin gene."

An updated Figure 3 is also provided in section A2.13. Kite Pharma does not believe that the environmental risk assessment needs to be updated.

Question 9

You describe the production of phoenix ECO cells. In this context you should state how hygromycin resistance and diphtheria toxin resistance are achieved. Are these resistance genes present on the specified pCRIPenv$^-$ and pCRIPgag$^-$2 plasmids? If so, where? Or have they been introduced on separate plasmids? If so, which ones? The origin and function of all component parts should be described in each case.

Re Question 9:

The hygromycin resistance gene was introduced into the cells using the pSV2HgM plasmid by co-transfecting it along the pCRIPenv- plasmid that contains the retroviral gag-pol polyprotein. The diphtheria toxin resistance gene (gpt) was introduced into the cells using the pGPT2E plasmid by co-transfecting it with the pCRIPgag-2 plasmid containing the ecotropic envelope. The resulting cell line was named Phoenix ECO.

"The hygromycin resistance gene within the pSV2HgM plasmid was derived from an Escherichia coli gene that confers resistance to the hygromycin B antibiotic (Bernard et al., 1985). This gene is driven by the viral SV40 promoter. The diphtheria resistance gene, named gpt, was cloned from Escherichia coli and confers resistance to hypoxanthine-aminopterin-thymidine medium when transfected into hypoxanthine phosphoribosyltransferase negative cell strains (Jasin et al., 1988). The use of selection markers facilitates the identification of positive clones expressing both the gag-pol polyprotein

and the envelope. None of the selection markers are incorporated in the retroviral particles produced by the Phoenix-ECO cell line."

References:

Bernard HU, Krämmer G, Röwekamp WG. Construction of a fusion gene that confers resistance against hygromycin B to mammalian cells in culture. Exp Cell Res. 1985 May;158(1):237-43. (Annex 1)

Jasin M, Berg P. Homologous integration in mammalian cells without target gene selection. Genes Dev. 1988 Nov;2 (11):1353-63. (Annex 2)

A2.13 has been updated with this information

Question 10

In A2.10 you state that **approximately** 1.2 x 10^9 transducing units (TU) are used to transduce the patient's T cells. You should state what TU range is used and what is the **maximum** quantity of TUs used? The environmental risk analysis should be based on the maximum quantity of TUs used.

Re Question 10: *As part of the current manufacturing process the transduction takes place at the Kite Pharma manufacturing facility in California. The volume of viral vector added to generate KTE-C19 is fixed, and the vector titer (TU/ ml) is known. 1.4 x 10^9 TU represents the maximum transducing units used per KTE-C19 lot. Kite Pharma applies a range of 1.3-1.4 x 10^9 Transducing Units per KTE-C19 lot. Please note that the final T cell product (KTE-C19 Final Product) does not contain any free retrovirus when transferred back into the EU and the clinical site in the Netherlands and as such no update to the environmental risk analysis is considered necessary.*

Section A2.10 has been updated accordingly.

Question 11

In A3.2 you describe testing the viral vector for the presence of replication- competent retrovirus (RCR), and give the limits of detection for the S+L assay (1 RCR/100ml RVV supernatant). You should also indicate the maximum quantity of RCR that could theoretically be present in a virus batch without being detected. You should also indicate what the abbreviation RVV means.

Re Question 11: *The detection limits for the S+L- assay is 1 RCR per 100 mL of retroviral vector (RVV) supernatant. However 300 mL of retroviral vector harvest supernatant was tested for RCR out of approximately 80 liters from a PG13-CD19-H3 Vector manufacturing campaign. Therefore the theoretical number of RCR particles per manufacturing campaign is less than 266 (~80 liters / 300 mL < 266 RCR particles). Additionally, 1 x 10^8 cells from the end of the PG13-CD19-H3 Vector production batch were co-cultured with human 293 cells in a test to look for RCR in the production cells, and no RCR-positives were found.*

Section A3.2 has been updated accordingly.

Question 12

In A3.2 of the application form you state the limit of quantification for the qPCR assay to detect GALV envelope DNA. This limit of quantification is expressed in copies per quantity of genomic DNA (10 copies of GALV env DNA per 200ng of genomic DNA). Please indicate how many cells this equates to, how many cells are used during the production of a batch of KTE-C19, and what quantity of cells are analysed in the case of patient material. You should specify how much GALV env DNA (i.e. how much RCR) can theoretically be present without being detected.

Re Question 12: *200 ng of DNA equates to approximately 30,300 cells. The KTE-C19 final product cell counts range from 100 - 200 million CAR-positive cells. Approximately 1.O X 10^6 patient PBMCS are collected at each time point and processed for*

RCR using qPCR assay. Theoretically the maximum amount of undetected RCR is less than 6600 particles. (~2.0 x 10^8 cells / ~3.03 x 10^4 cells < 6600 particles).

Section A3.2 has been updated accordingly.

Question 13

In A4.4 it is stated that 3 months after infusion the number of KTE-C19 cells fell to very low levels, and a frequency of <0.01% is reported. To what does this reported frequency relate? For example, <0.01% of total PBMCs (peripheral blood mononuclear cells), or <0.01% of $CD3^+$ T-cells?

Re Question 13: *Three months after infusion the number of KTE-C19 cells fell to very low levels with a frequency of <0.01% of total PBMCs (peripheral blood mononuclear cells). Section A4.4 has been updated accordingly.*

Question 14

You should back up assumptions/statements in the application with references to scientific literature or studies and supply this documentation with your application. There are no references in A2.13 (T cells are largely unable to produce infectious virions).

Re Question 14: *Section A2.13 has been updated with the appropriate reference as described below:*

Since human and murine retroviruses differ, it is highly unlikely that recombination with endogenous retroviruses will occur in human T cells. In addition, T cells are largely unable to produce infectious virions (Bear et al., 2012). Furthermore, T cells cannot survive outside of the human body.

Question 15

No personal information should be reported in A1.10 as this section will be made public if the permit is granted. You should delete the personal information from this section.

Re Question 15: *We have now deleted the names for all sites under A1.10 and also the names on page 4 of the Application form (Consent form).*

Question 16

You need to confirm that the number of patients in the study (table 1 in A1.5) is increased to 230. Is it correctly stated that in your hospital only pediatric ALL patients will be treated?

Re Question 16: *It is correct that in the PMC only children with ALL will be included, and that the numbers may increase to 50 (originally planned 25), which makes the total number of patients included in all studies mentioned in table 1 230 instead of 205.*

Question 17

In part A of the application form data are changd in A1.7, A1.8 and A1.10. You have to confirm that these changes were erroneously, and correct the data.

Re Question 17: *Please accept our apologies for these erroneously added changes; we have corrected them in the current version.*

Question 18

In A4.9 you refer to AMC guidelines on waste that may contain GMOs. You should also submit this document. All documents (including data, instructions, information material, guidelines, procedures and protocols) referred to in the application should be submitted. If, for example, you refer to a few pages of certain documents it is not necessary to send the entire document provided that this information can be read and understood in isolation. You should be aware that all information in the application form is in the public domain.

Re Question 17: *We erroneously referred to the AMC guidelines. We will handle according to the appendix-9 of the GMO-*

regulation of 2013. We have changed the text in A4.9 accordingly.

Question 19

In A1.4 you state that the permit is being requested for administration of the GMO and for the removal/processing of waste. For the sake of completeness, you should add the reception of the GMO as this also takes place in the PMC (and if applicable: taking samples from patients).

Re Question 19: *Wherever relevant, we have added that the application also covers receipt and thawing of the GMO and sampling of the patients.*

Question 20

In A1.10 you state where T cells are infused into the patient and where waste is processed. You should add where the GMO reception procedure, GMO thawing (and if applicable: taking samples from the patient) take place. In addition, for all activities you should also specify where (for example, in which department and in which building in the hospital) the activities take place. The application form states "*For clarification: for all activities, the location where they are to be performed should be stated.*"

Re Question 20: *In A1.10, we have now added details about the receipt of the GMO, the thawing and infusion of the T-cell product and the sampling of the patients. Receipt will be at the Cell Therapy facility of the UMCU, and thawing and infusion will take place in a patient room at the Princess Máxima Center. As discussed with* [redacted] *by phone on August 6th, no further specification of room number is necessary.*

In your letter to the AMC concerning IM-MV 16-004, which uses the same GMO, you had an additional remark that we considered relevant for our IM-MV 16-006 application as well. Therefore, we have addressed this remark in our application as well.

Additional remark (Question 20 in the reply on your letter of july 28th regarding IM-MV 16-004 by AMC):

You state in A2.10 that the half-life of GALV-pseudotyped retroviral vectors is less than 4 hours in the absence of stabilisers (Carmo et al. 2009 J Gene Med; 11: 670-678). You therefore use a half-life of 4 hours in calculating the virus reduction ratio. However, the half-life of GALV-pseudotyped retroviral vectors is between 8 and 10 hours in the presence, for example, of human serum albumin or FBS. The half-life in a culture medium also is about 8 hours (Carmo et al. 2009 J Gene Med; 11: 670-678). Therefore, a half-life of 4 hours during the production process is possibly too optimistic an assumption. If a half-life of 8 or 10 hours is used, the residual number of virus particles is not negligibly small. You should adapt your application accordingly and include the consequences of this in your environmental risk assessment.

Re Additional remark (Question 20 in reply by AMC): *Section A2.10 has been updated as described below:*

Retroviral vectors are well known to be unstable at physiological temperatures (Carmo et al, 2006; Higashikawa and Chang 2001; Carmo et al., 2008; Wikstrom et al 2004), with a reported half-life at 37°C ranging from about 2-4 hours. The half-life for -VSV-G, amphotropic and GALV-pseudotyped retroviral vectors were described, with similar results. Carmo and colleagues describe a half-life of less than 4 hours for a GALV-pseudotyped retroviral vector in the absence of stabilizers such as rHSA or 10% FBS at 37°C (Carmo et al., 2009).

Cell culture medium components in the PG13-CD19-H3 Vector preparation that could act to stabilize the vector are removed from the KTE-C19 manufacturing stream after completion of retroviral vector transduction by a wash step, designed to remove retroviral vector-related process impurities, prior to initiation of the cell growth and expansion step. This cell growth and expansion step is conducted in a serum-free, chemically-defined culture media with no known retroviral vector stabilizers. These data fit with the worst case scenario of a 4 hour half-life of the PG13-CD19-H3 Vector. This corresponds to a loss of vector transducing units of approximately 64-fold per day. During the KTE-C19 manufacturing process, T cells are grown at 37°C for a minimum of 4 days after completion of retroviral vector transduction and subsequent wash step. Incubation at that temperature for 4 days would therefore be expected to reduce the titer of non-replicative retroviral vector by more than 8 logs.

Kite Pharma does not believe that the environmental risk assessment needs to be updated.

Bijlages

aantal bijlages 2

Bevat de aangeleverde informatie vertrouwelijke gegevens?

- ○ ja
- ◉ nee

1. Nummer de bijlagen.
2. Verstuur de aanvullende informatie, inclusief bijlagen. Dit mag eventueel ook per mail.