Application form

Assessment of clinical research involving gene therapeutics in the Netherlands

-

Viral vectors

June 2016

Application form
Assessment of clinical study involving genetically modified viruses

Part A: Bio-safety aspects

A1. General information (Public section)
A2. Construction and composition (Public section)
A3. Production (Public section)
A4. Research description (Public section)
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A6. Conclusions about possible environmental effects (Public section)
A7. General (personal) information (Confidential section)

Part B: Patient-related aspects (*not included in this form*)

If you have any questions, please get in touch with the Gene Therapy Office (E-mail: rik.bleijs@rivm.nl, phone: +31-30-2747569).

June 2016

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Part A. Bio-safety aspects

This part of the application form provides the information needed for the Ministry of Infrastructure and Environment (IenM) to grant the necessary licenses.

All information provided in this form and the accompanying documentation constitutes part of the decision to be made and for this reason is in principle open to inspection by the public; the information will also be available for such public inspection during the procedure.

The applicant may ask for parts of the information provided to be kept confidential. In that case, the applicant must give reasons why the information is of a confidential nature as well as a convincing explanation that the lifting of confidentiality will adversely affect the applicant's competitive position. A publicly available summary of confidential information must be given, containing enough information for a clear general understanding of the application and in order to assess the risk analysis as described in the application and the decision. Confidential information must be included in a separate attachment marked 'confidential'.

An application does not need to be limited to the specific clinical protocol that the applicant wishes to perform. If there are no consequences for the risk analysis, the application can be drawn up with a wider scope, such as for a larger number of patients.

The aim is to draw up the final decision in such a way that several clinical protocols can be performed under it, using the information described in this application. Naturally these activities must be covered by the description of the experiment and the risk analysis provided. Before submitting such a broader application, you are advised to contact the GMO office for an informal discussion of the options.

The term 'test subjects' as used in this form means patients or volunteers taking part in the study.

Specific personal details of the contact person and the environmental safety officer are to be submitted by entering the required information under Section A7, General (personal) information. The submitted data in this appendix will be kept confidential and will thus not be made publicly available in accordance with the Personal Data Protection Act.

This Notification Form may contain some questions that are not relevant to your case. We would ask you NOT to answer in your notification any questions that are irrelevant to the activities for which you are applying.

Specific issues:

- Literature that is referred to has to be sent in together with the application form.
- Confidential information has to be marked as such and has to be sent in separately.
- A SNIF B (other GMO) form has to be completed and to be sent in as an electronic file in Word format.

A1. General application details

General information

A1.1. Application title:

Please give a descriptive title that provides sufficient information on the objectives and aspects relating to the genetic modification. The title should state the type of GMO(s) and insertion(s) used and the nature of the application(s).

Testing the safety and efficacy of JCAR017 in patients with aggressive B cell malignancies. JCAR017 product is comprised of autologous CD4+ and CD8+ T cells transduced via a self-inactivating (SIN) lentiviral vector to express a CD19-specific CAR comprising an scFv binding domain derived from the FMC63 murine CD19-specific mAb fused to the 4-1BB and CD3ζ chain signaling domains. JCAR017 CAR T cells are redirected toward recognition and lysis of CD19-expressing target cells including malignant cells.

A1.2. The objective of the study for which the application is submitted:

Provide a short description of the study's objective. For example: 'The objective of this Phase I clinical trial is to study the safety of the oncolytic adenovirus for the purpose of developing a new therapy to treat skin cancer.'

The objective of clinical studies with JCAR017 is to investigate its safety and efficacy for the purpose of developing this CAR T cell therapy for treatment of patients with aggressive B cell malignancies.

A1.3. Describe briefly the contents of the application, the objective of the study being aimed for and the intended application of the results

Provide a short description – of no more than half a page – of the genetically modified organisms (GMOs) applied, or of the GMOs that may thus be created, such as via recombination of genetic information between organisms or by integration of genetic material into a genome. Describe the expected action of the gene products of the transgenes and give an explanation of the biological mechanism. Also describe the scientific and public importance of the study, and state how the results of the study may be used in this context.

The information provided will be used as the basis for a brief description of the study in the decision.

The GMO (JCAR017) contains genetically modified T lymphocytes that are transduced ex vivo in a GMP manufacturing facility and then supplied to the clinical sites for infusion into the patient via intravenous route. The patient's own T cells are isolated from leukapheresis collection material in the manufacturing facility, genetically modified to express a CAR comprising an antigen-binding region of a CD19-specific monoclonal antibody linked to the 4-1BB and CD3ζ chain signaling domains, and then re-infused back into the patient where they are intended to expand and generate a persistent antitumor response. Of note, the SIN lentiviral vector transduction of T cells occurs in the US, and the JCAR017 product shipped to the clinical sites does not contain replication competent viral particles.

CD19 is a 95-kDa transmembrane glycoprotein expressed on more than 95% of B-cell malignancies (Ramos, 2014). CD19 is an attractive target for adoptive immunotherapy because, while present on most B-cell leukemias and lymphomas, it is not expressed on hematopoietic stem cells or on normal cells other than those of B-cell lineage (Sadelain, 2013).

B-cell malignancies represent a heterogeneous group of disorders with widely varying characteristics and clinical behavior and include B-cell lineage acute lymphoblastic leukemia (ALL) and B-cell non-Hodgkin's lymphomas (NHL). The morbidity and mortality associated with B-cell malignancies represent a significant unmet medical need.

CAR T cells have shown remarkable activity when directed against the pan-B-cell antigen CD19 with significant responses and durable remissions in patients with B-cell malignancies (Turtle, 2016a; Turtle, 2016b; Kochenderfer, 2015; Maude, 2014).

A1.4. Describe briefly the intended work.

State in chronological order which types of procedures will be carried out, and for which a license is being applied for (e.g. production, transport, storage and administration of the vector, observation of patients, sampling, transport, storage and processing of samples, waste treatment). Where applicable, also indicate for which part of the study a Contained Use (IG) permit applies, and provide the number of the GMO permit concerned.

The JCAR017 manufacturing process begins when autologous peripheral blood mononuclear cells (PBMCs) are obtained from the patient via standard leukapheresis collection procedures (no part of this application), and the PBMCs are shipped to a manufacturing facility for further processing to generate the JCAR017 cell product. Following receipt at the manufacturing facility, T cells are isolated ex vivo using commercially available magnetic beads. The cells are activated with a commercially available T cell activation reagent and then transduced with the SIN lentiviral vector (ZRX-014-LV) encoding the CD19 CAR. JCAR017 cells are expanded ex vivo to a therapeutic dose in a controlled bioreactor and harvested. The cell harvest is washed and formulated in a cryopreservation media. Following filling and labeling, the JCAR017 cell product is cryopreserved.

Information regarding the location of manufacturing operations is proprietary confidential information. Such information is provided in a separate Appendix marked "confidential". Publishing the abovementioned information would disclose confidential, trade secret information and give an advantage to other companies developing similar treatments.

JCAR017 Investigational Medicinal Product (IMP) will be shipped to the clinical site in a validated shipping container. Storage of JCAR017 IMP will take place in accordance with the GMO regulations as described in annex 9.3.2 (ODG). Immediately prior to the scheduled administration to the patient JCAR017 IMP will be thawed on site and administered to the patient via intravenous infusion in a hospital infusion area. The appropriate clinical site personnel will be trained in handling, administration, thawing and product accountability.

Any partially used or unused JCAR017 IMP and any supplies used in the preparation and administration process, including the IV administration set, must be disposed of in accordance with the GMO regulations (Appendix 'Registratie ggo-afval').

The license is being applied for the following procedures: receipt, storage, thawing and administration of the finished product JCAR017, sampling of patients, sample handling and waste disposal.

A1.5. Intended start and end date:

The decision must state a time period within which the procedures will be carried out, and so an end date must be given. The chosen end date will be included in the decision. It is possible to obtain an extension to the decision; please note, however, that any extension procedure must be completed before the decision end date has passed.

Intended duration of clinical JCAR017 program is Q4 2017 to Q4 2040. When the patients complete the study or discontinue will be conducted up for up to 15 years.

A.1.6. Do you want to keep other information confidential? If so, please specify in concrete terms how the release of the information would harm your competitive position.

Unless marked "Confidential", all the information contained in the notification and its appendices may enter the public domain when the notification is publicly processed and the decision is published.

For the sections marked "Confidential", you are requested to give a publishable summary that contains enough information to ensure a good general understanding of the notification. Furthermore, give a reason why certain information is marked "Confidential".

The structure, schematic diagram, sequence of lentiviral vector, plasmids, and regulatory elements, manufacturing process and specifications, including method description, protocol, detection limit, and acceptance criteria for the SIN viral vector and for the JCAR017 final product, are proprietary confidential information. Such information is provided in a separate Appendix marked “confidential”. Publishing the abovementioned information would disclose confidential, trade secret information and give an advantage to other companies developing similar treatments. A brief public summary is provided in those sections where the confidential Appendix is cited.

Details of applicant

Only the legal entity that has final responsibility for the work to be carried out may act as the applicant. This means that the applicant will normally be the Board (management) of the hospital (institution) where the treatment will be given. The license holder must be able to enforce compliance with the license regulations when carrying out the work. In order to do so, it is necessary for the employees involved in the clinical procedures to come under the authority of the license holder. For this reason, employees must be directly employed by the license holder. In those cases where an employee does not come under the authority of the license holder, such as where a treating doctor is part of a partnership that is independent of the license holder, an employment contract must be arranged for carrying out work under the license, such as through a zero-hours contract with the license holder. A contract must be concluded with the party or parties carrying out this work for non-clinical procedures that are not carried out in the institution in question, in such a way that final responsibility continues to rest with the license holder.

A1.7. Name of legal entity: Erasmus MC

A1.8. Chamber of Commerce (KvK) number: 24485070

A1.9. Visiting address of legal entity: Wytemaweg 80

A1.10. Postal code and town/city of location of legal entity: 3015 CN Rotterdam

A1.11. At which locations will the intended work take place?

Since the work applied for may only be carried out under the direct control of the license holder, it is only possible to carry out work at several locations if the license holder has full control of the way in which the work being applied for is carried out at all locations, in such a way that the license conditions are complied with. In that case you must state for each location what work will be carried out at which address and in which building. To clarify: you must state for all activities at which location they will be carried out. Apart from the location for the clinical activities with the GMO, you must also state the location or locations of laboratories in which activities with the GMO are carried out under the terms of this license application, such as procedures with patient samples.

In cases where central control is not possible, such as with a multi-center study, a separate application must be submitted for each location.

Receipt, storage, thawing and infusion of JCAR017 and waste disposal, sampling of patients and handling of samples will be executed in the Department of Hematology in the Erasmus MC.

Receipt and thawing of the GMO will take place in the stem cell laboratory. Administration of the GMO will take place in a patient room with restricted access at the hematology department at the Erasmus MC. Gathering of the waste that contained the GMO product or that has been in contact with the GMO product will be done at the hematology department in bins for specific hospital waste (UN 3291) according to the GMO regulations (Appendix 'Registratie ggo-afval'). Closed bins will be transported by the logistics department to the logistical exit point of the hospital where the bin(s) will be handed over to the company which will take care of the transport to the waste destruction company. Sampling of patients can take place at the department of hematology, at the outpatient department or any other department if necessary. No additional measures need to be taken for handling of samples since there is no additional risk compared to samples of any other patient.

Site:

Department of Hematology

Erasmus MC

Wytemaweg, 80

3015 CN Rotterdam, The Netherlands

A2. Construction and composition of the GMO

Virus from which the genetically modified vector was derived

A2.1. Which virus was used as the original virus in the construction of the GMO?

Where applicable, supply the full scientific name, a trivial name (e.g. the commercial name), the strain and the isolate used.

The JCAR017 genetic construct is delivered into T cells using a lentiviral vector originally derived from HIV-1 (human immunodeficiency virus, type 1). The sequences have been altered as described further below in sections A2.4 and A2.9. As is typical with lentiviral vectors, the JCAR017 lentiviral vector is pseudotyped with an envelope derived from VSV (vesicular stomatitis virus). Parts of the lentiviral vector genome contains transcriptional regulatory sequences from WHV (woodchuck hepatitis virus) and from TaV (Thosea aesigna virus). The promoter contains partial sequences from HTLV (human T cell leukemia virus).

A2.2. Describe how the identity of the original virus was determined.

The GMO to be administered is often based on a strain that was derived, sometimes by genetic modification, from the original virus. The properties of the original organism form the basis for the environmental risk assessment, which is why confirmation of the identity is important. Provide a description of the characteristics that determine the identity of the original virus. This may be based on bio-information analyses, such as sequence analysis, alignments or phylogenetic analysis. Information on the origins of the original virus, such as a micro-organism originating from the American Type Culture Collection (ATCC), may also be submitted.

The lentiviral vector system derived from HIV-1 is a third generation replication-defective self-inactivating lentiviral vector system that is typical of most third generation lentiviral vectors in use today (see Dull et al. Journal of Virology, 1998). The system consists of 4 plasmids (1 transfer, 3 helpers) used to make the lentiviral vector which have been identified by sequencing and database alignment.

These plasmids have been sequenced with four-fold coverage and sequences have been aligned to GenBank databases using BLAST in order to confirm identity of the original viral elements. The transfer and gag/pol helper plasmids utilize minimal cis elements derived from common lab strains of HIV-1 sequences. The transfer plasmid also contains a modified WPRE (woodchuck hepatitis virus post-transcriptional regulatory element) fragment, modified from the original woodchuck hepatitis virus (WHV) genome. These modifications are for the purpose of inactivating the partial open reading frame for X protein, normally potentially oncogenic, within the WPRE, for potential enhanced safety (Kingsman et al. Gene Therapy 2005). The Rev helper plasmid is derived from HIV-1. The envelope glycoprotein helper plasmid is derived from VSV (vesicular stomatitis virus) glycoprotein.

The description of how the identity was determined contains proprietary confidential information. Such information is provided in a separate Appendix marked “confidential”. Publishing the abovementioned information would disclose confidential, trade secret information and give an advantage to other companies developing similar treatments.

A2.3. What is the host range of the original virus?

Describe the hosts in which the original virus naturally occurs, also including hosts that serve as a reservoir. For each possible host, indicate the tissue and cell tropism.

HIV-1, the main viral system that the lentiviral vectors are derived from, has a host range of humans and some non-human primates (Cullen, HIV compendium, Los Alamos National Laboratories, 2001). The tissue and cell tropism of HIV-1 consists primarily of T cells, with evidence of tropism towards tissue resident macrophages and microglial cells (Clapham and McKnight, British Medical Bulletin, 2001).

A2.4. Provide relevant data on pathogenicity and possible attenuation and biological restrictions of the original virus.

What is the class of pathogenicity of the original virus? If this concerns an attenuated virus, the basis for attenuation must be described. If the organism is biologically restricted in another way, the grounds for the biological restriction must be substantiated.

Wild type HIV-1 is classified in pathogenicity class 3 according to the COGEM.
The lentiviral vector used as part of the JCAR017 process is based on HIV-1 however it has been modified to make it non-pathogenic by splitting the genome and removing most accessory genes (Dull et al. Journal of Virology, 1998), and by making the HIV-1 promoters self-inactivating (Zufferey et al. Journal of Virology, 1998). With these modifications there has never been a replication-competent lentivirus detected in the clinical history of lentiviral vectors (Cornetta et al. Molecular Therapy, 2011; Bear et al. Molecular Therapy, 2012).

A2.5. What are the pathogenic properties of the original virus and what are the available treatment methods?

Name the symptoms that are known or assumed to be caused by the original virus. Also indicate the treatment methods that are available to treat such an infection and provide an indication of the effectiveness of these treatments.

HIV-1 causes AIDS (acquired immunodeficiency syndrome) in humans. The virus is treated with anti-retrovirals, however the virus is never removed. AIDS is managed symptomatically (Simon et al. Lancet, 2006).

A2.6. What are the transmission routes of the original virus?

State all the observed and assumed transmission routes of the virus, and indicate the effectiveness of the transmission.

HIV-1 is transmitted by mucosa (sexual intercourse), by breast milk, and by blood (Simon et al. Lancet, 2006).

A2.7. How could the original virus survive outside the host?

State all survival options and the survival time of the original virus under optimal environmental conditions, and describe the factors that may be of influence.

HIV-1 can remain stable in blood in syringes at room temperature for 42 days (Abdala et al. Substance Use Misuse, 2000), and in blood and cerebrospinal fluid from autopsies for up to 11 days (Ball et al. Lancet, 1991).

The genetically modified viral vector

A2.8. Describe the 'original vector or vectors'.

Describe the 'original' vector, and indicate – with or without the help of bio-information analysis – in what way the original vector deviates from the original virus. For the application of a viral system (e.g. lentiviral particles), in addition to the viral (transfer) vector, you must also describe for example packaging and pseudotyping plasmids. Provide a description of the characteristics that determine the identity of these original vectors. This can be done on the basis of bio-information analysis, such as sequence analysis, alignments or phylogenetic analysis.

The lentiviral vector system derived from HIV-1 is a third generation replication-defective self-inactivating lentiviral vector system that is typical of most third generation lentiviral vectors in use today (see Dull et al. Journal of Virology, 1998). The system includes 4 plasmids (1 transfer, 3 helpers) used to make the lentiviral vector. The transfer plasmid contains only minimal *cis* components from HIV-1 necessary for the lentiviral life cycle. These components are: the 5'LTR and partial 3'LTR (for self-inactivation), the psi packaging sequence, splice donor and acceptor sites, and the Rev response element (RRE). The transfer plasmid also contains the WPRE (woodchuck hepatitis virus post-transcriptional regulatory element) fragment from WHV, and the transgene itself contains human sequences linked by the 2A cleaving sequence from TaV. The promoter for the transgene is a chimeric promoter containing the HTLV partial LTR sequence (the R element) and the human EF1alpha promoter.

The 3 helper sequences are summarized as follows: The Rev plasmid encodes the HIV-1 Rev accessory protein, driven by a human CMV promoter. The VSV-G plasmid encodes the vesicular stomatitis virus envelope glycoprotein, driven by a human CMV promoter, for pseudotyping of lentiviral vectors. The *gagpol* plasmid encodes the HIV-1 Gag and Pol proteins, driven by a human CMV promoter, providing the structural and enzymatic components of the lentiviral vector.

The descriptions of the transfer plasmid and 3 helper plasmids are proprietary confidential information. Such information is provided in a separate Appendix marked "confidential". Publishing the abovementioned information would disclose confidential, trade secret information and give an advantage to other companies developing similar treatments.

A2.9. Regarding the pathogenicity of the original virus, have certain properties of the 'original vector' been altered that would determine the pathogenicity of the original vector?

Here, an elaboration could be given of the modifications made to the 'original vector', such as replication deficiency, which cause attenuation of the pathogenic properties.

Modern lentiviral vector production systems are engineered to be self-inactivating, replication incompetent, and have been improved to reduce the risk of replication-competent lentiviruses (RCL) generation which may arise from recombination of split genome viral vector components (Dull, 1998). Although homology among split genome components have been significantly reduced some homology is unavoidable due to the current design of lentiviral vectors. All four plasmids used for vector production share homologous bacterial backbone plasmid sequences necessary for plasmid production. These are: ColE1 ori, f1 ori, Ampicillin resistance promoter and gene. All plasmids utilize a human CMV promoter which is 752bp long in the helper plasmids and 601bp long in the transfer plasmid. The Rev plasmid and the VSV-G plasmid share homology through the presence of the rabbit beta-globin intron (573bp) and rabbit beta-globin polyA sequences (523bp). Regarding the split genome viral vector components, all of the HIV-1 accessory genes (*tat, vif, vpu, vpr, nef, env*) have been removed except for the *rev* gene which is present on the Rev plasmid. The transfer plasmid and the GagPol plasmid share homology with the RRE (258bp) and a partial *gag* sequence (631bp). Following reverse transcription and integration into the target T cells, the partial *gag* sequence does not contain an active LTR promoter to drive its transcription due to the self-inactivating (SIN) design of the viral vector.

The potential that replication competent lentivirus (RCL) would be produced during the in vitro generation of virus stocks is very low. The risk is minimized during the production of

the lentiviral vector by using a split-genome third-generation system where the plasmids encoding the segments and genes required to form the viral vector are segregated onto separate plasmids: the envelope glycoprotein (not derived from a lentivirus) is on one plasmid, the *gag* and *pol* genes on another plasmid (derived from HIV-1), the *rev* gene on another plasmid (derived from HIV-1) and the transfer genome encoding the transgene on a separate plasmid (derived from HIV-1 but self-inactivating due to a deletion in the 3'LTR). These sequences are provided *in trans* via transfection of plasmids into the HEK-293T cell line which allows for only transient expression of these constructs during the viral vector production stage. The risk for RCL is even further reduced by retaining the Rev-dependence of the viral vector: Rev is required for export of the RNA genome transgene from the nucleus into the cytoplasm for protein expression and packaging. Since Rev is provided only *in trans* and since the Rev protein is not packaged in the virus the chance that a lentiviral RNA genome can continue its nuclear export in transduced cells is highly unlikely. Finally, the self-inactivating nature of the vector means that expression off of the LTR is significantly reduced due to the 3'LTR deletion and the absence of the HIV-1 *tat* gene (normally required for LTR-driven transcription).

Although the generation of RCL is unlikely, testing is performed for the presence of RCL at multiple locations in the manufacturing process, beginning with testing of the vector, followed by testing of the final gene-modified T cell product, and finally with patient monitoring after cell infusion.

A2.10. Describe the method of production of the clinical viral vector from the 'original vector or vectors'.

Answer this question preferably by using a diagram that describes the various production steps.

The production of ZRX-014-LV replication-incompetent lentiviral vector uses human embryonic kidney (HEK) 293T cells expanded from a Master Cell Bank (MCB) at a contract manufacturing organization for Juno Therapeutics. The vector is isolated from the supernatant of the 293T cells that have been transiently transfected with three helper plasmids and the transfer plasmid pZRX-014 that expresses a CAR with specificity for the B cell antigen CD19 and, via a T2A ribosome self-cleaving linker peptide, expresses a truncated form of the human epidermal growth factor receptor (EGFRt).

The MCB has been characterized and tested to ensure it does not produce potentially pathogenic viral particles as well as for other safety testing.

The method of production is proprietary confidential information. Such information is provided in a separate Appendix marked "confidential". Publishing the abovementioned information would disclose confidential, trade secret information and give an advantage to other companies developing similar treatments.

A2.11. Describe the coding genes and the regulatory sequences present in the vector and in the DNA inserted into the vector.

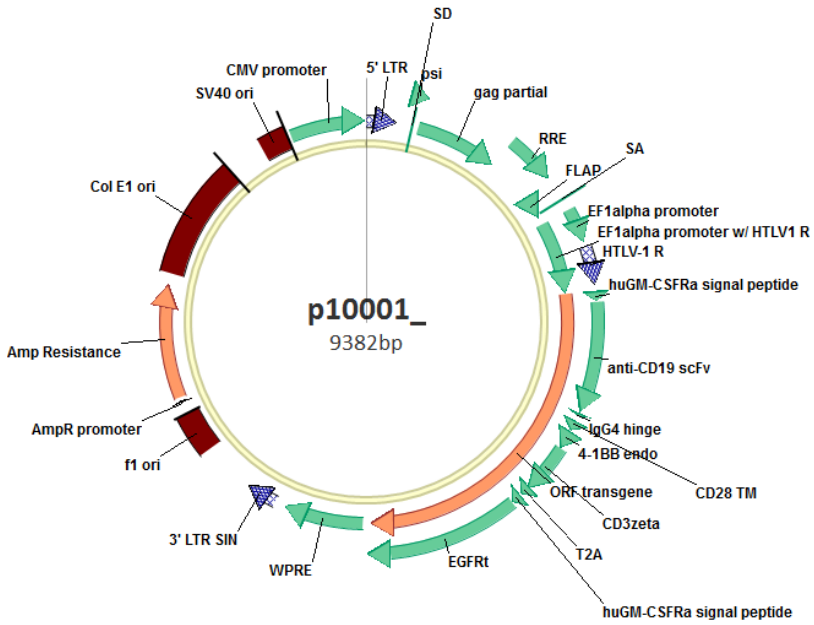
A full description must be provided of the inserted or deleted genetic material, also discussing the functions of the sequences, for example:

- *regulatory sequences, such as promoter, terminator, and enhancer sequences;*
- *structural genes;*
- *in case of insertion of a transgene: the function of the coded proteins in the donor organism (the donor organism is the organism from which the gene originally was isolated or in which it occurs naturally) and the expected function in the test subject;*
- *in case of deletion: the function of the deleted genetic material in the original organism;*

- *in case of point mutation(s): the effect of the point mutation or mutations on the function of the modified genetic material in the original organism and the expected function in the test subject;*
- *whether the vector or the DNA inserted into the vector contains elements of which the origin or function is unknown.*

The transfer plasmid houses the CD19-specific CAR fragment linked to EGFRt with a self-cleaving T2A peptide: driven by an EF1α HTLV-1 hybrid promoter. Only sequences from the 5'LTR to the 3' LTR are packaged into the viral vector. The open reading frame encodes:

- An N-terminal leader peptide of the human GMCSF receptor alpha chain signal sequence to direct surface expression of the CAR
- CD19-specific scFv derived from the IgG1 murine monoclonal antibody FMC63
- Human IgG4 hinge and human CD28 transmembrane region
- 4-1BB costimulatory element
- Cytoplasmic tail of human CD3zeta
- T2A linker peptide
- An N-terminal leader peptide of the human GMCSF receptor alpha chain signal sequence to direct surface expression of EGFRt
- EGFRt, a truncated non-functional human epidermal growth factor receptor type I transmembrane polypeptide. Domains I and II, as well as the cytoplasmic tail of EGFR have been deleted resulting in the truncated form, EGFRt, which contains only domains III, IV and the trans-membrane domain (Wang et al. Blood 2011). This truncation is such that EGFRt retains the binding site of cetuximab (Erbitux) within domain III, allowing for selection, detection and ablation of engineered cells (if necessary). Deletion of domains I and II prevents binding to its natural ligands Epidermal Growth Factor (EGF) and Transforming Growth Factor alpha (TGFα). Deletion of the cytoplasmic tail renders EGFRt void of signalling activity.



Transfer Plasmid:

Element	Origin	Function
5’ LTR	HIV-1	LTR containing R and U5 elements from HIV-1
Psi	HIV-1	Packaging signal
Splice donor (SD)	HIV-1	Initiate splicing of vector RNA
gag partial	HIV-1	cis components required for packaging
RRE	HIV-1	Rev-responsive element
FLAP	HIV-1	pre-integration complex
Splice acceptor (SA)	HIV-1	Terminate splicing of vector RNA
EF1alpha promoter with HTLV-1 R	Human and HTLV-1	promoter (and intron/enhancer) for driving expression of the transgene
huGM-CSFRa signal peptide	Human	Signal sequence
Anti-CD19 scFV	Mouse domais; synthetic linker peptide	binding domain of CAR
IgG4 hinge	Human	Spacer of CAR
CD28 TM	Human	Transmembrane domain of CAR
4-1BB endo	Human	Cytoplasmic domain of CAR
CD3zeta	Human	Cytoplasmic domain of CAR
T2A	Thosea Asigna virus	Self-cleaving linker polypeptide
huGM-CSFRa signal peptide	Human	Signal sequence
EGFRt	Human	marker of transduced cells
WPRE	Woodchuck hepatitis virus	enhance viral RNA transportation
3’ LTR SIN	HIV-1	LTR; self-inactivating (SIN).
f1 ori	f1 phage	replication origin
AmpR promoter	E. coli	Promoter for Ampicillin resistance.
Amp Resistance	E.coli	Ampicillin resistance
ColE1 ori	ColE1 plasmid from E.coli	Replication origin
SV40 ori	Simian Virus 40	Replication origin
CMV promoter	Human	promoter

Element	Origin	Function
	CMV	

The full sequence starting with the 5'LTR through the gag partial, including all intervening sequences, match the HIV-1 genome without any gaps. All of the intervening sequences are commonly used in third generation lentiviral vectors. The intervening sequences do not add any additional environmental risk to the considerations outlined in this document.

The descriptions of the coding genes including intervening sequences are proprietary confidential information. Such information is provided in a separate Appendix marked "confidential". Publishing the abovementioned information would disclose confidential, trade secret information and give an advantage to other companies developing similar treatments.

A2.12. Provide a molecular characterization of the genetically modified viral vector.

A sequence analysis must be supplied for all inserted or deleted sequences, so that the precise location of the modification and, in case of insertion, the number of copies present and the orientation of the insertion cassette can be determined. The sequence to be submitted must contain the region of the intended modification as well as the recombination sequences used, also including the flanking sequences of the genome of the original organism. In the case of biological unrestricted GMOs, newly created fusion ORFs must be described and subjected to a bio-information analysis.
The complete genome of the viral vector must be characterized on a molecular level. This may be done according to a sequence analysis or a Southern blot analysis. During the production of a GMO, naturally occurring processes may lead to unintended modifications in the genome. These modifications may affect the fitness of the ultimate GMO and, thus, the results of the environmental risk assessment. Observed anomalies in the genome compared with the expected sequences, such as unexpected deletions, mutations or recombinations, must be described and interpreted. Phenotypic data may be used to support the data on the molecular characterization.
The sequence of the complete genome does not need to be submitted. A bio-information analysis must be carried out for the sequences and the annotated results must be presented. For more information, please refer to the COGEM advisory report CGM/130227-05.

Molecular characterization/confirmation of the identity of the transfer plasmid and the packaging plasmids:

As stated in A2.2, the lentiviral vector system comprises 4 plasmids (1 transfer, 3 helpers) used to make the lentiviral vector. These plasmids have been sequenced with four-fold coverage and sequences have been aligned to GenBank databases using BLAST in order to confirm identity of the original viral elements. The detection limits are based on homology to known reference sequences provided in the section A2.2 in confidential Appendix. The validation acceptance criteria are greater than or equal to 98% identity to reference over a minimum of 534bp read. The two forward and two reverse sequence reads must have a QV of ≥ 20. The transfer plasmid and two of the helper plasmids sequences were confirmed to be 100% identical to the known reference sequences. The third helper plasmid had an undetermined base located outside of the helper functional region. The uncalled base was determined not to affect helper function and does not impact the environmental assessment.

Molecular characterization/confirmation of the identity of the lentiviral vector:

The identity of the CAR was confirmed by Sanger Sequencing of the vector RNA. Purified vector RNA is extracted, reverse-transcribed to complementary DNA (cDNA) and a double-stranded DNA sequencing template is generated by PCR amplification of the CAR region

using target specific fusion primers. To determine the nucleotide sequence, thermal cycling is performed with chain-terminating fluorescently-labeled nucleotide base analogs. The nucleotide order of the resulting RT-PCR product is then determined. The resulting nucleotide sequence is compared to the known CAR sequence. The CAR sequence is evaluated for 100% homology to the known, reference sequence. The CAR sequence has been confirmed to be 100% identical to the reference.

In addition, sequencing of the viral vector RNA genome was performed to characterize the region predicted to insert into the host cell genome, spanning the 5' Long Terminal Repeats (LTR) to 3' LTR. For this characterization Next Generation Sequencing (NGS) was performed. For NGS, the reference sequence provided to the laboratory includes the entire vector sequence between 5' LTR and 3' LTR, including the functional and/or identifying sequences that encode CAR, T2A, and EGFRt. The vector reference sequence is a theoretical region spanning the expected genetic material that would be enclosed in a viral vector.

The vector sequence evaluated by NGS exhibits 99.98% identity to the reference sequence. A low frequency variant (28.64%) was identified. The substitution impacts neither cis-regulatory elements nor CAR sequence. The potency and function of the vector lot is not impacted by the substitution, as evidenced by performance of the vector material in transduction assays and a JCAR017 scale down model. The functionality of the vector and integrated sequences are not impacted by the low level mutation therefore there is no impact to the environmental risk assessment. Also the substitution identified in the low frequency variant is not expected to impact the likelihood of a (theoretical) RCL event. Any potential environmental impact/risk is mitigated by RCL testing strategies for vector and drug product release.

Finally, RNA transcript analysis of JCAR017 (the transduced cell product) has been performed and no high confidence mutations (> 10% of RNAseq reads) have been detected in the CAR sequence itself. Based upon the RNA transcript analysis, no high confidence mutations have been observed within the CAR transgene.

Additional information regarding molecular characterization and confirmation of the identity of the lentiviral vector are proprietary confidential information. Such information is provided in a separate Appendix marked "confidential". Publishing the abovementioned information would disclose confidential, trade secret information and give an advantage to other companies developing similar treatments.

Molecular characterization/confirmation of the identity of the transduced cells:
The stability of the transgene insert has been demonstrated using the Southern blot method of genetic sequence identification. The purpose of this assay is to confirm the presence of unrearranged vector sequences within genomic DNA of cells transduced with lentiviral vector supernatant.

Genomic DNA, isolated from cells transduced with the lentiviral supernatant, was digested with restriction enzymes. The resulting DNA fragments were isolated via agarose gel electrophoresis. Probes were then generated via PCR or restriction enzyme digestion. The DNA for the test and control samples, ZRX-014 transfer plasmid, was hybridized with the radiolabeled probe and exposed to X-ray film to produce a signal denoting the

presence/absence of the sequence in question. The analysis demonstrated a band consistent with the predicted fragment size.

The ZRX-014-LV vector relies on the HIV-1 reverse transcriptase (RT) enzyme for converting its single stranded RNA genome into double stranded DNA before integration into target cells. For this reason the integrated viral vector sequence, including the transgene, is influenced by the fidelity of the HIV-1 RT enzyme. As reviewed in Sanjuan et al. (Journal of Virology, Oct 2010, p.9733-9748), the mean mutation rate of HIV-1 is thought to be $2.4x10^{-5}$ substitutions per nucleotide per cell infection. With the theoretical viral vector sequence size described in the confidential attachment to Part A, this would translate to a very low, but non-zero, incident of theoretical mutations of the integrated viral vector sequence. The low likelihood of these mutations would not change the environmental risk assessments discussed in this application.

The annotated sequence for the ZRX-014-LV vector is proprietary confidential information. Such information is provided in a separate Appendix marked "confidential". Publishing the abovementioned information would disclose confidential, trade secret information and give an advantage to other companies developing similar treatments.

A2.13. Describe the origins of the cells/cell lines in which the original viral vector is cultivated. Also indicate which of the genetic components of the cell could possibly cause complementation or recombination.

When answering this question, elaborate on the characteristics of all cell lines to be used. Describe which cell types this concerns as well as their origins (e.g. human kidney epithelial cells).Also discuss the possibility of the genetic material in the cells/cell lines causing a certain interaction with the original vector, such as by complementation or recombination.

Manufacture of ZRX-014-LV utilizes a 293T cell line. The 293T cell line is a highly transfectable derivative of human embryonic kidney 293 cells, and contains the SV40 T-antigen (DuBridge et al. Molecular and Cellular Biology, 1987). This cell line is used for transient transfection of DNA plasmids encoding the JCAR017 lentiviral vector system. The T-antigen allows for the amplification of plasmids containing the SV40 origin of replication, once transfected, thus resulting in high lentiviral vector titers. The 293T cell line has been widely used for retroviral production, gene expression and protein production (Rio, 1985; DuBridge, 1987). The original embryonic kidney cells were immortalized using sheared adenovirus genome (Graham and Smiley, Journal of General Virology, 1977). Unlike other stable producer cells that contain retroviral sequences, this cell line contains no genetic modification besides the SV40 and adenovirus sequences. For this reason any chance of interacting with the original vector is highly unlikely (Pear, 1993). Furthermore, RCL testing is performed on end of production cells to test for any replication competent lentivirus formation. With these viral vector systems there has been no evidence of replication competent virus formation (Cornetta et al. Molecular Therapy, 2011; Bear et al. Molecular Therapy, 2012).

The Master Cell Bank for the 293T cell line was tested with the following virus assays and determined to be negative for each test: Transmission EM For Viruses And Retroviruses, Simian Virus 40 (SV40), Product Enhanced Reverse Transcriptase (PERT) Assay, In-Vitro Viral Assay, Bovine Viral Assay, Porcine Viral Assay, Amphotropic or Xenotropic Retrovirus, Replication Competent Retrovirus With The GAL-V Envelope Proteins, In-Vivo Viral Assay, Murine Viruses Known To Infect Mouse Tissue, Human T-Cell Leukemia Virus I, Human T-Cell Leukemia Virus II, Human Immunodeficiency Virus I, Human

Immunodeficiency Virus II, Hepatitis Virus B, Hepatitis Virus C, Human Herpes Virus 6, Human Herpes Virus 7, Human Herpes Virus 8, Cytomegalovirus, Epstein-Barr Virus, Human Parvovirus B19, Bovine Viral Assay, In Vivo Detection Of Adventitious Viruses in Eggs, Test For The Presence of Inapparent Viruses in Guinea Pigs.

A2.14. Summarize the data in a diagram ('map') of the genetically modified organism. Also indicate any relevant helper sequences that may possibly be present.

Present clear maps of the genetically modified organism, such as plasmid maps, showing all the constituent parts of the vector. In this schematic depiction, the construction of the GMO must be clearly indicated.

See the figure in response to A2.8 , A2.11, A2.12, and in confidential Appendix.

A2.15. Indicate the degree to which the host range of the genetically modified viral vector has been or may be altered, relative to the original virus.

When answering this question, provide an argument that elaborates on the host range, host specificity and the tissue and cell tropism of the genetically modified viral vector, relative to the original virus. Also consider any modifications that were made in order to create the original vector.

The original virus has a host range towards humans and some non-human primates, as described in section A2.3. This tropism has been altered as follows:
Instead of the HIV-1 envelope, the JCAR017 lentiviral vector has been pseudotyped with the VSV glycoprotein envelope, therefore altering the host range to that of VSV (vesicular stomatitis virus) which includes humans, horses, cattle, pigs, mules, sand flies, grasshoppers and rodents (Nunamaker et al. Journal of Medical Entomology, 2003; Lichty et al. Trends in Molecular Medicine, 2004). Due to pseudotyping with VSV glycoprotein the cell tropism has also expanded from the original virus (T cells and macrophages) to any cell type that expresses sufficient levels of the receptor, LDL-R (Finkelshtein et al. PNAS, 2013).

Note that the viral transduction is performed ex-vivo and that the transduced cell suspensions infused in the patients does not contain replication competent virus particles (see section A3.2 in confidential Appendix).

A2.16. What physiological (including pathogenic) effects may be caused by the genetically modified viral vector; and what are the available treatments?

Indicate which physiological processes may occur following the application of the GMO in the host.

A comparison must be made between its possible pathogenic properties and those of the original virus. In particular, the pathogenic properties that may be created specifically by the GMO should be considered.

Viral Vector

As described in A2.9 and A2.15, ZRX-014-LV, the lentiviral vector used in the production of JCAR017 is replication-defective and self-inactivating. Testing is performed for the presence of RCL at multiple locations in the manufacturing process, beginning with testing of the vector, followed by testing of the final gene-modified T cell product, and finally with patient monitoring after cell infusion. RCL testing is also done in the drug product prior to patient administration. Due to the wide tropism of the VSV-G envelope, in the unlikely event that residual replication incompetent viral vector is present, transduction and genomic insertion of exposed cells cannot be excluded. However human serum complement is capable of inactivating VSV-G (DePolo, 2000). While highly unlikely, antiretroviral medications could

be used for these scenarios. Because the majority of viral sequences from the original HIV-1 have been eliminated from the viral vectors the pathogenic effects of these unlikely scenarios are unknown.

JCAR017 (Transduced T-cells)

Although this section requests a description of the biological activities that can be caused by the genetically modified viral vector, ZRX-014-LV vector is not applied *in vivo* as an advanced therapeutic investigational medicinal compound. As described in A3.2, the investigational medicine product JCAR017 contains the genetically modified autologous T cells and does not contain replication competent viral vector particles.

ZRX-014-LV vector mediates integration of the CAR sequence into the host T cell chromatin, with subsequent expression of the CAR on the T cell surface. The anti-CD19 CAR T cells are expanded and infused back to the patient as JCAR017. JCAR017 T cells can recognize and eliminate CD19 expressing target cells (Abramson, 2017). Consequently, physiological and pathological effects observed in patients are the consequence of the engineered T cells not the viral vector itself.

Immunogenicity.
It is possible that the transduced JCAR017 may be immunogenic, and that the patients will have an immune response directed against the scFV; this has not had clinical consequences in previous anti-CD19 CAR T cell trials (Kochenderfer, 2015). If an immune response to the cells occurs, it is possible that the cells will be rejected.

Immunoglobulin depletion.
Transient or prolonged host B cell depletion is also a potential risk with JCAR017 cells, since normal B cells also express CD19 (Davila, 2013; Grupp, 2013). This is expected to resolve when the JCAR017 cells are cleared. Patients that develop JCAR017 related B cell aplasia are at increased risk of infections and may require periodic infusions with immunoglobulin to prevent infections.

Transformation/insertional mutagenesis.
There is a risk that people who receive gene transfer may develop new tumors derived from their genetically modified cells. This risk is primarily associated with viral gene transfer vectors that integrate into the cellular DNA where they may dysregulate genes controlling proliferation. Transformation has not been observed following adoptive T cell transfer in hundreds of cancer and HIV patients receiving gamma retroviral modified T cells treated on multiple protocols at many academic centers.

Theoretically, the insertion of the DNA sequence of the replication-deficient lentiviral vector into the T cells is non-directional and therefore, bears the risk of insertional mutagenesis. However, three factors prevent such insertion events from being directly carcinogenic:

i. First, retrovirus vector insertion is almost uniformly monoallelic.
ii. Second, some gene insertions may trigger differentiation or apoptosis, or otherwise reduce the survival probability of the affected cell clone.
iii. Third, a single insertional mutation is, to the current knowledge, not sufficient to develop a malignant phenotype (Baum et al. Molecular Therapy, 2004).

No cases have been described in the literature in which malignant transformation of a mature CAR genetically modified T cell has been demonstrated. Furthermore, there is no risk of

insertion into the germline as mature CD4+ and CD8+ T cells will be used as starting material.

If a malignant transformation would occur, this could be treated using chemotherapy and/or radiation therapy.

Uncontrolled T cell proliferation.
JCAR017 cells could proliferate without control of normal homeostatic mechanisms. In the context of this protocol it is possible that T cells will proliferate in response to signals from the malignant tumor or normal B cells. This could be beneficial or harmful depending on the extent of proliferation. If any subject develops excessive JCAR017 cells accumulation, corticosteroids will be administered to eradicate the infused cells. This has not been observed to date with JCAR017.

Risk of Tumor Lysis Syndrome (TLS) related to cytoreductive chemotherapy or CAR T cells.
TLS as a consequence of pre-conditioning chemotherapy or anti-CD19 CAR T cell treatment has not been observed in the previous anti-CD19 CAR T cell trials. The risk of TLS is dependent on the disease and burden of disease, but in most cases, the risk will be low. Patients will be closely monitored both before and after chemotherapy and JCAR017 infusions. Should signs of acute TLS occur patients will be treated as per the standard of care.

Cytokine Release Syndrome (CRS)/Macrophage Activation Syndrome (MAS).
CRS has been observed in patients after treatment with JCAR017 (Abramson, 2017). Patients with clinical responses exhibited some levels of CRS that ranged from mild to severe consisting of fever, hypotension, capillary leak, hypoxia or other symptoms.

Cytokine production is associated with the activation, expansion and cytolytic function of T cells and for JCAR017 T cells. Therefore, some degree of CRS may be a desired clinical outcome. Premature or early intervention with anti-cytokine therapy may therefore abrogate the anti-tumor efficacy of JCAR017. However, in case of severe CRS requiring treatment, tocilizumab (an anti-IL-6-receptor antibody) therapy has been utilized with effective management of CRS and successful JCAR017 T cell expansion (Abramson et al. J Clin Oncol, 2017).

Neurotoxicity.
Another class of adverse events that has been observed with anti-CD19 CAR T cell therapy is central nervous system toxicity including hallucinations, delirium, aphasia, confusion and somnolence. These toxicities range from mild to severe and in extreme cases may require ventilatory support for airway protection. Neurological toxicity generally emerges within the first week after anti-CD19 CAR T cell infusion, and resolves within a few days. Similar to CRS, the neurological toxicities reported with anti-CD19 CAR T cell therapy to date have generally been medically manageable with supportive care, tocilizumab and corticosteroids, and have resolved (Davila 2014, Maude 2014, Kochenderfer 2015, Gardner 2017).

A2.17. Indicate the possible transmission routes of the genetically modified viral vector.

Provide all the observed and assumed routes of transmission. Indicate the degree to which the transmission of the GMO could be made easier by helper functions, or by the presence of replication-competent virus. Furthermore, indicate the degree of impact on transmission

due to the modification and the cells in which the GMO was cultivated or those infected with the GMO. Make a comparison with the original virus.

Viral Vector

The viral transduction is performed outside of the Netherlands. The final cell suspension product (JCAR017) does not contain replication competent viral particles (see A3.2).

Even if a very low number of infectious replication incompetent viral particles would be present in the JCAR017 cell product, the vector is replication-deficient and could only integrate once and no further spreading is possible. In addition, any remaining replication incompetent vector will be inactivated by the complement component of human serum after administration to the patient or accidentally to an unintended recipient (Welsh, 1975; Welsh, 1976). Possible transmission routes of the replication incompetent vector would be blood-borne and mucosal exposures.

JCAR017 (Transduced T-cells)

The predicted habitat of the genetically modified cells (the JCAR017 cell product) is only the patient from which the cells were derived. Factors that may allow release of JCAR017 cells outside the restricted host are blood tests and accidental injury. If these occur, there is no risk to the environment as JCAR017 does not survive in the environment, and is rapidly inactivated by standard means of disinfection.

Any released JCAR017 cells cannot be transmitted by air, but could be transferred through accidental injection or contact with broken skin. Medical personnel (or other individuals), if exposed to JCAR017 through accidental injection of minimal numbers of JCAR017 cells, would eliminate the cells via their immune system and not experience adverse effects beyond a normal immune reaction. Thus no lasting negative consequences are expected in this case.

A3. Production of the GMO

A3.1. State under whose responsibility the production of the GMO is carried out.

Answer:

- O Production will be under the responsibility of the applicant and forms part of this license application.
- O Production will be by and under the responsibility of the applicant but does not form part of this license application:
  - O A separate application for production will be submitted for contained use
  - O Reference is made for the production to an existing license for contained use: (State here the number of the relevant GMO license)
  - O Production will be under the responsibility of third parties. If production is in the Netherlands, please state the number of the relevant GMO license. Please state if production is outside the Netherlands.

Celgene Corporation will be the sponsor of the clinical trials with JCAR017 in Europe. The production of the GMO will be done by Juno Therapeutics Inc. in the USA. The production will take place outside the Netherlands (see also A1.4).

A3.2. During which steps of the production process does quality control take place, which test methods are used and how are the tests carried out?

Give an overview of the production process of the GMO and describe the points in the production process at which quality control takes place. State which controls are carried out, the sensitivity of the tests and which methods are used for the controls.

The JCAR017 manufacturing begins when autologous peripheral blood mononuclear cells (PBMCs) are obtained from the patient via standard leukapheresis collection procedures (not part of this application), and the PBMCs are shipped to a manufacturing facility for further processing into the JCAR017 cell product. Upon receipt at the manufacturing facility, T cells are isolated ex vivo using commercially available magnetic beads. The cells are activated with a commercially available T cell activation reagent and then transduced with the replication incompetent lentiviral vector (ZRX-014-LV) encoding the CD19 CAR. JCAR017 cells are expanded ex vivo to a therapeutic dose in a controlled bioreactor and harvested. The cell harvest is washed and formulated in a cryopreservation media. Following filling and labeling, the JCAR017 cell product is cryopreserved.

Within the JCAR017 manufacturing process and product, impurities can be categorized as process- or product-related. Process-related impurities include materials utilized or added during product manufacturing. The strategy for risk assessment for each process impurity is to estimate a safety margin derived from estimated permitted daily exposure generated from toxicology assessment and process reagent clearance modeling studies. Consequently, all reagents that pose potential safety risks are subjected to proper testing and control.

Process-related impurities are extrinsic to JCAR017 and the leukapheresis products. They are substances added to, or encountered during, the manufacturing process. Process-related impurities may be generated during JCAR017 manufacture, formulation, or storage. JCAR017 process-related impurities include residual selection and activation beads, replication incompetent viral vector, and media supplements.

Regarding replication incompetent viral vector, excretion of replication incompetent vector used to manufacture JCAR017 (“shedding”) by the patient is not likely to occur as the transduction of T- lymphocytes occurs ex vivo with a replication incompetent vector in a

GMP facility and minimal replication incompetent vector remains in the JCAR017 given to patients. Minimal residual replication incompetent vector is ensured in JCAR017; during the JCAR017 manufacturing process, replication incompetent vector is: (1) used up during the cellular transduction process, (2) removed from the product stream during media perfusion in the cellular expansion steps, and (3) diluted and removed by a series of cell washing and formulation steps. In addition, lentiviral replication incompetent vector has poor stability under the cellular culture and expansion steps (37C).

Although the packaging cell line and replication incompetent vector design make the generation of RCLs unlikely, extensive testing is performed at multiple levels to confirm the absence of RCL, including screening of the packaging cell line and replication incompetent vector product, testing of the final gene-modified T cell product, and with patient monitoring after cell infusion. All testing results of the packaging cell line and replication incompetent vector are confirmed to be negative for RCLs prior to use for manufacturing of the gene-modified T cell product.

The COGEM Advice CGM/090331-03 formula (COGEM formula) to assess the viral reduction ratio is provided in the confidential Appendix. A conservative viral reduction ratio of 0.2 is calculated per the JCAR017 values provided in the formula. This result is considered conservative as it does not include the wash effects of perfusion within the expansion operation, believed to further reduce any risk of RCL. In addition it uses a maximum value for the number of viral particles added of $8.0x10^8$. The conservative viral reduction ratio 0.2 estimates that there may be low levels of residual vector particles in the transduced T cell product. The estimated low level of residual vector particles pose low risk to the environment in that the vector is a third generation, replication incompetent vector which will not propagate in the environment. Should low levels of residual replication incompetent viral vector be present in the transduced T cells, transduction and genomic insertion of exposed cells cannot be excluded. However human serum complement is capable of inactivating VSV-G (DePolo, 2000) therefore infectious viral shedding is unlikely.

Product-related impurities are either present in the starting material or generated during manufacturing of the drug product cells. Product-related impurities are assessed individually for risk based upon known safety profiles for autologous cell therapy products and related-research. They may be generated during JCAR017 manufacture, formulation, or storage and include non-viable cells, platelets, red blood cells (RBCs), and residual non-T cells such as tumor cells and stem cells.

The ZRX-014-LV replication incompetent vector will be manufactured for use in GMP manufacture at a contract manufacturer. The transfection cell line is characterized at the contract manufacturer, including viability, cell number, and safety testing. Controls and testing for the manufacture of the lentiviral replication incompetent vector include raw material testing, in-process and release testing. These analytical release tests include replication incompetent lentiviral titer, identity (transgene sequence), microbiologic testing (including mycoplasma, endotoxin, sterility), and viral safety performed by the replication incompetent vector manufacturer.

Although quality control is established throughout all unit operations, the bulk of testing is performed at release stage. Specification testing at the release stage includes testing for Identity, Viability, Potency, replication incompetent Vector copy number, Endotoxin, Sterility, Mycoplasma, and RCL.

Following are summaries of assays and test methods for RCL testing.

qPCR Assay

Attribute	Test Method	Specification Acceptance Criteria	Limit of Detection/ Limit of Quantification
Replication-Competent Lentivirus (RCL) (VSV-G Analysis)	qPCR	⩽ 5 copies/50 ng gDNA	5 copies/50 ng gDNA (LLOQ)

Co-Cultivation Assay

Attribute	Test Method	Specification Acceptance Criteria	Limit of Detection/ Limit of Quantification
Replication-Competent Lentivirus (RCL)	Co-Cultivation with C8166 cells	Not detected	100 infectious units per $2x10^7$ cells

Description of Replication-Competent Lentivirus Test Methods

Replication-Competent Lentivirus qPCR Assay
The presence of replication-competent lentivirus (RCL) are detected using a PCR-based assay for the vesicular stomatitis virus G DNA (VSV-G) sequence in cryopreserved drug product. In this method, sequence-specific primers are used to detect VSV-G DNA sequence, as a surrogate marker for any replication competent lentivirus present in a sample.

Genomic DNA from drug product and a lot-matched untransduced sample (obtained at a point in the manufacturing process prior to transduction) are isolated using a commercial kit. This genomic DNA is used as template in a real-time PCR reaction using Taqman chemistry to amplify VSV-G. A DNA standard curve from VSV-G plasmid DNA is used to quantify the amount of VSV-G, while DNA extracted from the untransduced sample is used as a negative control. Additionally, this procedure incorporates beta actin as a reference gene whose detection serves as both a DNA isolation and PCR process control.

RCL Co-Cultivation Assay

For the detection of RCL, JCAR017 drug product cells are co-cultivated with C8166 cells over five passes. The supernatants from the amplification phase are applied to fresh C8166 cells during the indicator phase. The C8166 cell line is well characterized as a permissive cell line for lentivirus. Any RCL that is present or produced during the co-culture is detected in a subsequent p24 ELISA assay.

The detailed description of the manufacturing process and release testing is proprietary confidential information. Such information is provided in a separate Appendix marked "confidential". Publishing the abovementioned information would disclose confidential, trade secret information and give an advantage to other companies developing similar treatments.

A3.3. Which criteria are imposed on a batch of the GMO before it is released for the application in question?

State which criteria are used to reject a batch.

Release testing is performed as outlined in A3.2. Please refer to the confidential attachment for a more detailed list of release criteria. JCAR017 cell product is tested to confirm the absence of detected RCL via the PCR-based assay described in A3.2 in order to release the product for administration to the patient. In addition, after administration of JCAR017, results from additional RCL testing via a Co-Cultivation assay are used to further confirm the absence of RCL in the JCAR017 product.

A4. Description of the research

Administration

A4.1. How many test subjects will take part in the study?

Here, the maximum number of test subjects to be treated should be indicated. This number can be higher than the intended number of test subjects. Please be aware of the fact that the number you enter limits the permit. For example, if you indicate that there will be 50 test subjects, then no more than 50 can be included in the study.

A maximum of 200 patients with B cell malignancies are expected to take part in studies with JCAR017 in the Erasmus MC.

A4.2. Describe how the batch containing the GMO will be transported and prepared for administering to the test subjects.

Describe how, after delivery, the GMO preparation will be transported to the hospital pharmacy for example. Also describe the way in which the GMO preparation will be handled, as well as the conditions under which they will be handled, in order to get the preparation ready to be administered. If this handling will be carried out under an existing permit for Contained Use (IG), the number of the GMO permit concerned must be stated. In addition, describe the way in which the GMO preparation will be transported to the test subject.

JCAR017 Investigational Medicinal Product (IMP) will be shipped to the clinical site in a validated shipping container according to annex 1 of the GMO regulation. Transport within the institute will take place according to the GMO regulations. JCAR017 IMP will be thawed on site in the patient room and administered to the patient via intravenous infusion. The thawing process will be initiated once the bedside nurse provides notification that the subject is ready. The box that contains JCAR017 IMP cartons will be removed from the LN2 shipper. The carton containing the JCAR017 vials will be placed on a protective barrier pad, opened and thawed at room temperature for approximately 20-25 minutes.

After the thaw, JCAR017 will be prepared by inserting a sterile syringe needle into the port of the JCAR017 vial and withdrawing the target volume for the required dose into the syringe. The prepared dose will be immediately delivered to the bedside nurse for the administration of JCAR017.

The clinical site personnel will be trained in handling and administration, thawing and product accountability procedures.

A4.3. How will the GMO preparation be administered to the test subject?

State via which route and how the GMO is administered. Also state what aspects may affect the safety of human beings and the environment.

Administration of the GMO will take place in a patient room with restricted access at the hematology department at the Erasmus MC. The JCAR017 will be administered intravenously to the patients, slowly over 1-5 minutes.

Trained study staff will administer the JCAR017 intravenously using standard medical precautions for immunosuppressed patients. Patient protective isolation will be as per the institutional standards and policies. Even though JCAR017 is not associated with disease and is not pathogenic, exposure to the product will be restricted to the patient. The personnel handling the product vials containing JCAR017 will follow the appropriate gowning

procedure for cell therapy wearing disposable gloves and an apron. No extra safety measures will be taken for JCAR017.

Patients will be admitted into the hospital to receive the JCAR017 infusion in a standard patient room suitable to be decontaminated with disinfectants. 70% Ethanol will be used for regular disinfection on used surfaces. In case of a spill, the surface will be treated with 1000 ppm Chloride.

Central venous access such as a port or a peripherally inserted central catheter is required for the administration of JCAR017 and for the hospitalization treatment period. Catheter care, per institutional guidelines, should be followed.

JCAR017 therapy can lead to severe adverse reactions for the patients receiving it in the clinical studies. The most significant toxicities in patients who received CAR T cells have been severe cytokine release syndrome (sCRS) and neurotoxicity (Abramson, 2017).

The risk for health care personnel is low. Contact with the IMP via skin or other sensory organs after a spillage or during waste disposal does not present any clear risk as the product would quickly lose viability in the environment and even if the transduced cells were to remain viable for several hours, for any adverse events to occur that are related to the genetic modification, the route of administration would have to be via direct infusion into the blood stream. Otherwise the risks would be no different to exposure to a non-genetically modified cell.

Needle-stick injuries can occur for any procedure involving the needle-based infusion of a medicine and in a clinical environment, this risk is mitigated by permitting only medically trained professionals to have access to the IMP and that have taken the necessary safety measures to prevent such an injury e.g. safety procedures, protective clothing, etc. However, the risk of the genetically manipulated material causing harm in this way is negligible due to the following factors:

- Any transfer of gene modified cells received from a needle stick injury is likely to be very low when compared with an intentional infusion
- Medical professionals and healthcare workers in general are highly unlikely to be immune compromised and therefore any infused cells are unlikely to persist as healthy immune-competent individuals would be expected to promptly reject the allogeneic T-lymphocytes
- Transduction of the cells is carried out ex vivo and requires specific in vitro experimental conditions and direct activation of the target cells (mature T-lymphocytes) but as minimal replication incompetent viral particles remain in the IMP (section A3.2 in confidential Appendix), in vivo transduction is highly improbable
- Human exposure would be minimized in a contained environment (i.e. a small, controlled area within a hospital/clinic)

The risk for the general environment is negligible. Human T cells require complex solutions, environmental, and physical controls in order to survive outside the human body. Without these controls and in the general environment human T cells will not survive. Also, excretion of replication incompetent vector used to manufacture JCAR017 (“shedding”) by the patient is not likely to occur as the transduction of T- lymphocytes occurs ex vivo with a replication incompetent vector in a GMP facility and minimal replication incompetent vector remains in the JCAR017 given to patients.

A4.4. Which doses will be administered and at what time points during the study will they be administered?

Indicate which doses of the GMO will be administered. Also provide an administration diagram, showing at what times they will be administered.

JCAR017 will be administered at single flat doses not exceeding 1.5 x 10^8 JCAR017 transduced cells/infusion. Subjects who have responsive disease, including disease that is no longer progressing and is stable, but who have not achieved a complete response (CR) following JCAR017 infusion or those who have achieved a response and have progressed disease (PD) or relapse may receive an additional dose of JCAR017 if dose is available. Minimum interval between administrations will be 28 days (time elapsed between JCAR017 administration and first response assessment). All toxicities must have resolved to Grade 2 or less prior to commencing a subsequent treatment. If additional doses of JCAR017 are not available, repeat leukapheresis may be allowed after discussion with the Sponsor.

The description of the number of lentiviral replication incompetent vector particles that will be used for T cell transduction is proprietary confidential information. Such information is provided in a separate Appendix marked "confidential". Publishing the abovementioned information would disclose confidential, trade secret information and give an advantage to other companies developing similar treatments.

A4.5. What other medication will be administered to the test subject that may possibly affect the GMO to be administered?

Any medication that would affect the GMO and possibly also the environmental risk assessment should be named here. If, for example, a vaccination study is conducted, this may also include challenge with the wild-type virus.

Patients will receive lymphodepleting chemotherapy treatment prior to infusion with JCAR017. Such treatment does not affect the GMO.

Patients should be premedicated with 500 to 650 mg paracetamol PO and 25–50 mg diphenhydramine hydrochloride (PO or IV) 30–60 minutes prior to JCAR017 infusion, with no effect expected on the GMO based on their mechanism of action.

No medication will be administered during JCAR017 infusion, however in case of severe toxicity concomitant medications such as tocilizumab or steroids may be used.

Administration of high lymphotoxic doses of steroids as treatment of CAR T cell related severe cytokine release syndrome (sCRS), has resulted in rapid reversal of the clinical symptoms but has also shown to abrogate CAR T cell expansion and persistence (Davila 2014). The impact of lower doses of steroids on CAR T cell remains nevertheless unknown. Treatment guidelines recommend tocilizumab, which has not demonstrated effect on CAR T expansion to date, as first-line treatment, keeping steroids for patients unresponsive to tocilizumab.

Sampling

A4.6. Describe which of the samples taken from the test subject may contain GMOs.

Provide an overview of the samples and indicate whether GMO material is expected to be present in them.

Samples from subjects enrolled in clinical studies will be collected for up to 15 years following the last infusion of JCAR017. Samples will be analyzed by PCR for viral envelope sequences, the detection of which would represent potential evidence of replication-competent lentivirus (RCL). Due to integration of the replication incompetent viral vector into the host genome, the sequences will be present in transduced cells during the duration

that the cells persist following infusion. However, the patient samples are not expected to contain free replication incompetent viral vector, as the JCAR017 finished investigational drug product is theoretically unlikely to contain replication incompetent viral particles as demonstrated in the Section A3.2 in this form and the confidential Appendix, and any potential remaining replication incompetent viral vector particles in the product would have a high likelihood of being inhibited/inactivated by the complement component of human serum after administration to the patient (Alvarado et al. Journal of Veterinary Diagnostics Investigation, 2002; Tesfay et al. Journal of Virology, 2014).

A4.7. Describe the method of sampling and how the samples will be subsequently processed.

In answering this question, also indicate how transmission of the GMO during sampling and testing will be prevented. For the subsequent processing, indicate what physical restrictions apply. In case the work concerned will be carried out in the Netherlands but is not part of the current permit application, then refer to the permit for work under Contained Use and provide the number of the GMO permit this concerns.

Sampling of blood, tumor tissue and bone marrow will be performed according to the local site procedures by experienced laboratory personnel and nursing staff. GMO may be present in all patient samples collected.

To avoid possible spread of the blood, the blood draw will be performed with a closed tube system and used materials (ie. needle) will be disposed as specific hospital waste (UN3291). At various time points after JCAR017 final product infusion, approximately 50 mL of blood sample will be drawn from the patients to perform local and central laboratory assessments.

Samples will either be determined directly, stored for future use or transported to the external laboratories located in the US. Local analysis, processing, and storage will be done at a standard diagnostic laboratory of the center. Use of classified local laboratories is not required as the risk of GMO release to the environment is negligible.

Sampling, transportation of samples within the hospital, storage and processing will be done according to standard hospital procedures as the samples are expected to contain minimal level of residual replication incompetent viral particles (see A2.10 and A4.6) and any genetically modified cells present in the samples do not represent a specific safety concern (see A2.17 and A5.2 – A5.5). They will be treated as regular samples coming from patients.

The blood samples will be drawn according to pre-specified schedule of each trial. Blood will be handled according to institutional guidelines for biohazardous material.

Anti-CD19 transduced T cells, like human T cells, are easily inactivated outside the host by inappropriate media, or exposure to low pH, higher temperatures (>50°C), pasteurization (60°C for 10h), and microwave.

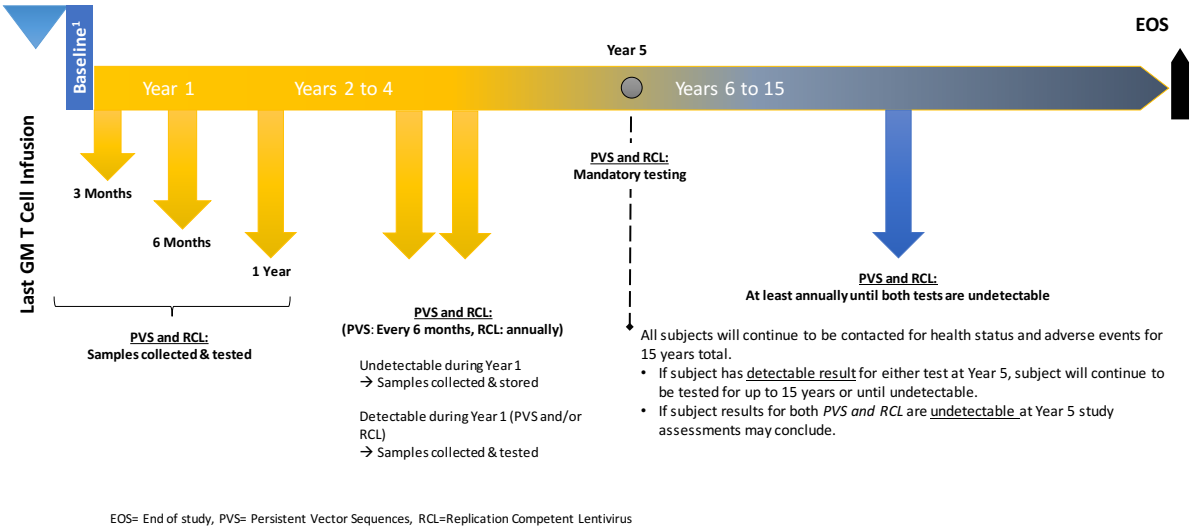
Cells present in the JCAR017 product are easily killed by lipid solvents, alcohol and disinfectants.

At site, 70% Ethanol will be used for regular disinfection on used surfaces. In case of a spill, the surface will be treated with 1000 ppm chlorine.

A4.8. How is the GMO preparation detected after being administered?

State, if applicable, when GMO components or are detected during or after administration and why detection at that particular moment of the test is regarded as important. Describe the nature of the samples that are tested, the method used and the detection limit that can be achieved.

Samples from patients enrolled in the clinical studies will be obtained for up to 15 years following the last infusion of JCAR017.Samples will be analyzed by PCR to confirm the absence of viral envelope sequences that would be indicative of replication-competent lentivirus (RCL).



EOS= End of study, PVS= Persistent Vector Sequences, RCL=Replication Competent Lentivirus

RCL qPCR Assay

The assay is designed to determine the level of VSVG envelope (VSVG-E) sequences present in the test sample using a quantitative PCR (Q-PCR). The method for detecting VSVG-E uses real time qPCR. The adequacy of the amount of test sample material analyzed is assessed by a second probe and primer set for human apolipoprotein B (ApoB) gene sequences. Samples of genomic DNA (gDNA) isolated from peripheral blood are provided for the assay. The level of detection for the assay is > 10 copies per 0.2ug DNA.

In the event that a sample was deemed positive by this assay, a duplicate sample would be submitted for repeat qPCR. If the duplicate sample was found to be positive, banked peripheral blood mononuclear cells (PBMCs) will be submitted for confirmatory cell based analysis.

Information regarding the qualification of this assay is proprietary confidential information. Such information is provided in a separate Appendix marked “confidential”. Publishing the abovementioned information would disclose confidential, trade secret information and give an advantage to other companies developing similar treatments

qPCR transduced T cell assay

The assay is designed for the quantitative determination of JCAR017 replication incompetent vector specific WPRE primer using a quantitative polymerase chain reaction (q-PCR) assay. The adequacy of the amount of test sample material analyzed is assessed by a second probe and primer set for human albumin (ALB) gene sequences. Samples of genomic DNA (gDNA) isolated from peripheral blood are provided. The level of detection for the assay is 2.5 copies/reaction (12.5 copies/ug).

Information regarding the qualification of this assay is proprietary confidential information. Such information is provided in a separate Appendix marked "confidential". Publishing the abovementioned information would disclose confidential, trade secret information and give an advantage to other companies developing similar treatments.

Waste management

A4.9. Give an overview of the nature and quantity of the waste produced and describe how the waste will be disposed of.

State which waste flows can be distinguished. State which waste flows may potentially contain the GMO and how the GMO is prevented from being released into the environment via the waste flows.

Any partially used or unused JCAR017 IMP (material remaining in vials used for administration at infusion sites), the vials, the absorbent barrier pads, any supplies used in the preparation and administration process, including the IV administration set, that contain the GMO or have been in contact with the GMO, must be disposed of in accordance with the GMO regulations (Appenix 'Registratie ggo-afval'). Since the waste from patient sampling will not harbor any extra risks, no additional measures will be taken compared to samples from any other patient and will be disposed of as specific hospital waste (UN3291). Closed bins will leave the patient room and will be transported to the logistical exit point of the hospital where the bin(s) will be handed over to the company which will take care of the transport to the waste destruction company (ZAVIN). The biohazard waste disposal will be registered in the investigator site files.
For cleaning and disinfection of the patient room after use will no additional measures need to be taken since there is no additional risk compared to any other patient room. 70% Ethanol will be used for regular disinfection on used surfaces. In case of a spill, the surface will be treated with 1000 ppm chlorine.

A5. Environmental risk analysis

Environment-related information originating from earlier experiments

A5.1. Describe the results originating from earlier (pre-) clinical studies with the GMO and which are important for the environmental risk assessment.

In answering this question, you should elaborate on results that were achieved using an identical or similar GMO, if these are relevant for the environmental risk assessment of the present application. Important data include data on shedding, duration of latent presence of the vector/GMO, transmission of the vector/GMO, and possible interaction with other micro-organisms (including viruses). For each of these results, describe the trial set up (e.g. doses used, method of administration, detection test and detection limit) and the relevance to the work in the present permit application.

PLAT-02 Study

A Phase 1/2 trial (Study PLAT-02 sponsored by the Seattle Children's Research Institute [SCRI], Seattle, WA) is currently being conducted to evaluate the safety, tolerability, pharmacokinetics (PK), and antitumor activity of a CD19 CAR T-cell product (hereafter referred to as SCRI-CAR19v1) in pediatric and young adult subjects with relapsed CD19+ B-cell ALL. SCRI-CAR19v1 uses the same construct as JCAR017, and was initially

manufactured using the same manufacturing process, so the data from the Phase 1 portion of the study are relevant to the assessment of JCAR017. Early safety results from the Phase 1 portion of Study PLAT-02 were used to guide dose selection for the Juno JCAR017 study.

In the Phase 1 portion of the study, a total of 43 subjects with relapsed CD19+ B cell ALL were treated with SCRI-CAR19v1 at dose levels ranging from 0.5×10^6 to 1×10^7 CAR T cells/kg (Gardner 2017). The majority of patients had 2 or more relapses and 28 of the 43 had a history of at least one prior allogeneic hematopoietic stem cell transplantation. All subjects received lymphodepletion chemotherapy prior to SCRI-CAR19v1 intravenous infusion: 27 received cyclophosphamide alone, and 14 received flu/cy.

No RCL has been detected in any SCRI-CAR19v1 drug product or patient samples in PLAT-02 study to date.

TRANSCEND NHL001 Study

The Juno sponsored TRANSCEND NHL001 is an open-label, multicenter, Phase 1 study to determine the safety, pharmacokinetics (PK), and antitumor activity of JCAR017 in adult subjects with R/R DLBCL and MCL. The study is being conducted in the US and patients are currently being enrolled.

The study is designed with 3 types of dosing groups. The dose-finding (DF) groups will evaluate and refine the dose and schedule of JCAR017 needed for safety and optimal antitumor activity in disease- specific cohorts. Dose-expansion (DE) groups will further assess the efficacy and safety of JCAR017. The dose-confirmation (DC) group will further evaluate the efficacy and safety of JCAR017 at the recommended regimen in the DLBCL cohort. The Primary Analysis Set will consist of at least 75 subjects from the DLBCL cohort treated at the recommended regimen that have been followed for at least 6 months or until death, disease progression, or withdrawal from study.

As per the cutoff presented at the ASCO Annual Meeting, June 2-6 2017, 67 DLBCL patients have been treated: 36 patients at a dose level of $5x10^7$ cells (single or double dose schedule), 19 patients at a dose level of $1x10^8$ cells (single dose), 5 not yet evaluable, and 7 received non-conforming product (Abramson 2017). Major safety findings include cytokine release syndrome (CRS), neurotoxicity (NT) and hematological disorders, including neutropenia, thrombocytopenia, and anemia. No fatal outcome from CRS or NT was reported (Abramson 2017).

No RCL has been detected in any JCAR017 drug product or patient samples in any study to date.

In vitro/ in vivo Relevant Results

Nonclinical data

Nonclinical studies of human autologous CAR T cells are necessarily limited due to the nature of the agent and the absence of cross-reactivity of JCAR017 to CD19 from non-human species (ie, mice, cynomolgus monkeys). For this reason, no in vivo safety studies of JCAR017 have been conducted in animals, and pharmacology studies have been limited to evaluating the anti-tumor activity in immune deficient mice.

Vector design

JCAR017 uses a replication defective lentiviral vector for generation of the autologous human T cell product. As currently proposed, autologous patient T cells will be transduced with a genetically-engineered lentiviral replication incompetent vector to express a CD19-specific CAR comprising an scFv binding domain derived from the FMC63 murine CD19-specific mAb fused to the 4-1BB and CD3ζ chain signaling domains.

A number of safety features have been incorporated in generating JCAR017. The viral genome has been split onto several plasmids to minimize the risk of generating a self-replicating vector by recombination, the deletion of enhancer/promoter sequences from the long terminal repeats (LTRs) to generate so-called self-inactivating (SIN) vectors, and VSV-G pseudotyping, which restricts the host range.

Replication competency

A third-generation self-inactivating (SIN) lentiviral vector, which encodes the CAR domains described above, is used to transduce the subjects' T cells. Additionally, the replication incompetent vector encodes a truncated human epidermal growth factor receptor (EGFRt) that is expressed separately from the CAR on the cell surface and serves as a non-functional marker for analytical detection of transduced T cells. Based on results in animal models (Paszkiewicz, 2016), EGFRt could potentially also be used for ablation of transduced cells.

Third-generation replication incompetent lentiviruses have several built-in safety features that minimize the risk of generating replication-competent wild-type human HIV-1 recombinants. Typically, LVs are generated by trans-complementation whereby packaging cells are co-transfected with a plasmid containing the vector genome and the packaging constructs that encode only the proteins essential for LV assembly and function (Matrai, 2010). The development of self-inactivating lentiviral vectors markedly improves lentiviral safety as it reduces the likelihood that replication competent retroviruses will originate in the vector producer and target cells, hampers recombination with wild-type HIV in an infected host, and reduces the likelihood that cellular coding sequences located adjacent to the vector integration site will be aberrantly expressed by abolishing the intrinsic promoter/enhancer activity of the HIV-1 LTR, (Zufferey, 1998; Matrai, 2010). The transcriptional inactivation of the LTR in the SIN provirus should also prevent mobilization by replication-competent virus upon subsequent infection with wild-type HIV-1 (Matrai, 2010). As a result, SIN lentiviral vectors have minimal risk of horizontal and/or vertical LV transmission, including to persons that handle or administer the gene therapy product, those involved in patient care, relatives and others.

Pseudotyping

The p10006 VSV-G plasmid encodes the vesicular stomatitis virus envelope glycoprotein, driven by a human CMV promoter, for pseudotyping of JCAR017 lentiviral replication incompetent vector. No replication-competent lentivirus has been detected in patients samples collected to date, indicating that there is no evidence of intact virus in treated subjects. Theoretically, should virus be present in administered product, integration into non-target cells would require distribution and infection, which would be dependent on the ability of the cells to be infected by virus. VSV-G uses the low density lipoprotein receptor (LDLR) for cellular infection (Finkelshtein, 2013). A review of on-line databases (Genotype-Tissue Expression [GTEx] and Human Protein Atlas) indicates ubiquitous protein expression across human tissues that would indicate the potential for infection.

VSV's natural hosts are cattle, pigs, horses and other mammals and their insect vectors (Hastie, 2013). Infection of these animals with JCAR017 would not lead to a productive infection due to

the design of the product and the marked species specificity, which restricts replication only in cells that originate from their natural host or from closely related species. This species specificity has hampered the development of animal models for HIV-1-induced disease and has necessitated the use of other lentiviruses as models for HIV pathogenesis, vaccine production, and drug development (Clements, 1996).

Shedding

The absence of detectable replication-competent lentivirus in drug product and in treated patients, along with the incorporate of SIN vector design and use of multiple plasmids for cell transduction, indicates negligible risk of environmental excretion of replication competent lentivirus from treated patients (Schambach, 2013).

Conclusions

Although nonclinical studies have not been conducted with JCAR017 due to the absence of cross-species activity, the available data indicate negligible environmental risk. This conclusion is based on the replication incompetent vector design, absence of detected replication-competent lentivirus in patients, and low theoretical risk should replication competent lentivirus be released into the environment.

Risk analysis

This is the most important aspect of the whole application!

Give a detailed assessment of the expected effects of the GMO on human health and the environment on the basis of the answers to the above questions and in accordance with Appendix II of EU Directive No. 2001/18/EC and the corresponding guidance notes of the European Commission (2002/623/EC). Please take into account any direct, indirect, immediate and delayed effects of the GMO on human health and the environment.

A risk analysis should be carried out for each GMO included in this notification, as well as for combinations of the GMOs, if any. The risk analysis must cover the effects of the GMOs that are due to interactions between the GMOs and the environment(s) where they are introduced or where they may end up under the present activities. The effects in question are those which are relevant to safety to human health and the environment. Section A6 of this form describes those aspects which must at least be taken into consideration.

The risk analysis should include at least the aspects mentioned in Annex 1 of this form. The risk analysis includes the following sections, which should be given in the same order as shown below (see questions A5.2 – A5.5):

1. *List of the likely adverse effects;*
2. *Estimate of the likelihood of these effects actually taking place;*
3. *Evaluation of the risks and an estimate of the severity of the effects, based on Items 1 and 2 above. The severity can be estimated by comparing it with the severity assigned to similar risks, such as for example the effects that occur with non-GMOs in similar situations ('baseline principle');*
4. *If you have concluded in Point 3 that the risk is high, you are requested to examine what measures can be used to mitigate the risk;*
5. *Final conclusion of the risk analysis, stating the risk management measures that will be employed, and a conclusion as to the acceptability of the risks when these measures are put into operation.*

A5.2. State which potentially harmful effects may be linked to exposure of human beings or the environment to the GMO.

Describe here the effects on human beings and the environment that could occur as a consequence of the use of the characteristics of the GMO described in earlier sections of this application. The aim here is identifying the hazards; the following questions deal with the chance that these hazards will actually occur.

Possible adverse effects related to JCAR017 final product (genetically modified T cell)

The genetically modified T cells are subject-specific and do not survive outside the subject. The cells are not pathogenic and do not persist or replicate in the environment. Therefore, no adverse effects on the environment are expected.

In the unlikely event of the provision of anti-CD19 CAR T cells to an unintended human recipient (through accidental injection), the engineered T cells would be recognised as non-self cells and therefore eliminated through the individual's innate (i.e. complement mediated lysis and phagocytic cells) and adaptive immune system (Chira et al. 2015; Welsh et al. 1975; Welsh et al. 1976). Adverse effects would be limited to a normal immune reaction to non-self cells, and no specific adverse effect related to the genetic modification of the cells is expected.

There is a theoretical possibility that the engineered anti-CD19 CAR T cells could persist if transmitted to an immunocompromised individual. In this highly unlikely case, the theoretical adverse effects would be the same as the possible adverse effects in subjects which are described in A2.16 (immunogenicity, immunoglobulin depletion, insertional mutagenesis. uncontrolled T-cell proliferation, cytokine release syndrome).

As described in A2.11 the EGFRt lacks the extracellular Domains I and II preventing the binding of its natural ligands, as well as the entire cytoplasmic tail rendering the EGFRt void of any signalling activity. No adverse effects on the environment or individuals exposed to JCAR017 are expected.

Possible adverse effects related to free replication incompetent vector particles

The level of free replication incompetent vector particles in the JCAR017 final product is considered to be low. After administration to the subject, any potential remaining replication incompetent viral particles would be inactivated by the complement component of human serum (Welsh, 2975; Welsh, 1976).

Regarding replication incompetent viral vector, excretion of replication incompetent vector used to manufacture JCAR017 ("shedding") by the patient is not likely to occur as the transduction of T- lymphocytes occurs ex vivo with a replication incompetent vector in a GMP facility and minimal replication incompetent vector remains in the JCAR017 given to patients. Minimal residual replication incompetent vector is ensured in JCAR017 as during the JCAR017 manufacturing process, residual replication incompetent vector is: (1) used up during the cellular transduction process, (2) removed from the product stream during the cellular expansion steps, and/or (3) removed over the course of a series of cell washing and formulation steps. In addition, replication incompetent lentiviral vector has poor stability under conditions such as temperatures used in the cellular culture and expansion steps carried out at 37C.

Additionally, although the packaging cell line and replication incompetent vector design make the generation of RCLs unlikely, extensive testing is performed at multiple levels to confirm the absence of RCL, including screening of the packaging cell line and replication incompetent vector product, testing of the final gene-modified T cell product, and patient monitoring after cell infusion. All testing results of the packaging cell line and replication incompetent vector are confirmed to be negative for RCLs prior to use for manufacturing of the gene-modified T cell product.

The COGEM Advice CGM/090331-03 formula to assess viral reduction ratio is provided in the confidential Appendix. A conservative viral reduction ratio was determined; the result is considered conservative as it does not include effects of certain aspects of the expansion operation, believed to further reduce any risk of RCL. The conservative viral reduction ratio is a higher value than number of replication incompetent viral particles added to the JCAR017 manufacturing process.

In the event of accidental injection of JCAR017 final product into unintended recipients, any theoretical remaining infectious replication incompetent viral particles would likely be inactivated by the complement in human serum. Even if an infectious replication incompetent particle would survive long enough to infect a cell, the replication incompetent vector is replication deficient and could only integrate once, and no further spreading is possible. No adverse effects would be expected due to a single or very few integration events.

Possible adverse effects related to the theoretical formation of RCL

In the highly unlikely event of RCL being present in the JCAR017 final product or formed in the subjects, a recombinant virus encoding the CAR transgene could be spread from cell to cell and also potentially to other individuals. In case of such an event, the viral particles would not survive in the environment outside of the body, as described for the parental virus.

Since there is neither RCL nor significant free replication incompetent particles expected, there is no possible harmful effects related to the absence of RCL and free replication incompetent particles that we can describe.

A5.3. State according to which scenario the GMO can disperse from the test subject into the environment.

State in your answer the scenarios in which the GMO may disperse in the environment. Explain the level of risk that spread will actually occur. Also describe in your answer whether the number of test subjects and/or the dosage to be administered affects the risks to be identified.

Scenarios that can lead to spread of the JCAR017 cell product (genetically modified T cells)

As described in section A5.2 the level of free replication incompetent vector particles in JCAR017 final product is considered to be low. The likelihood of release of JCAR017 from the subject is minimal except in case of accidental injury such as bleeding.

Since the JCAR017 cell product consists of genetically modified autologous T cells and the genetic modification introduced do not affect the host range, the habitat of the cells is restricted to the subject from which the cells were derived.

In the case of accidental release of JCAR017 cell product, the transduced cells cannot persist outside of the subject for long periods and maintain viability in the ambient environment (except for in appropriate cell culture conditions in the laboratory). Since the cells are sensitive to even moderately harsh conditions, it is highly unlikely that spread would occur in the environment and the genetically modified cells would be quickly rendered non-viable by the prevailing conditions.

The possible routes for any released anti-CD19 CAR T cells to be transmitted to unintended recipients is through accidental injection or contact with broken skin, or via exposure to the mucous membranes of the eyes, nose or mouth. However, in this unlikely case, JCAR017 would be eliminated through the person's immune system. Only in immunocompromised individual is there a theoretical risk that the JCAR017 cells could persist, and in this case they would not be expected to spread further. The risk of widespread infection is considered negligible.

In summary the anti-CD19 CAR T cells cannot be a source of further transduction, since replication defective lentiviral vectors integrate their genetic content into the T-cell genome without transferring virus-derived coding sequences.

Scenarios that can lead to spread of free vector particles

As described in A5.2, the level of free replication incompetent vector particles in the JCAR017 cell product is considered to be low. After administration to the subject, any potential remaining replication incompetent viral particles would be inactivated by the complement component of human serum. The risk of shedding or release of infectious replication incompetent viral particles from the subjects is therefore considered negligible.

During the JCAR017 manufacturing process, apheresis-derived material is subjected to selection of CD4+ and CD8+ T cells to high purity. The JCAR017 manufacturing process is designed to selectively activate and expand T cells. Even if small numbers of monocytes, which are precursors to both macrophages and dendritic cells, were present in the starting apheresis material, they would be cleared by the selection step and subsequent unit operations. A substantial reduction of monocytes after T cell selection is achieved, confirming that carrying macrophages or dendritic cells forward in the process is low risk. Additionally, macrophages and dendritic cells are susceptible to lysis over freeze and thaw cycles. Therefore, additional reduction of these cells following cryopreservation during the process is expected. Additionally, any CD14+ cells present prior to expansion would be outcompeted by the T cell populations during ex-vivo expansion. Both Macrophages and Dendritic Cells generally have poor replicative capacity during the ex-vivo expansion relative to the CD4+ and CD8+ T cells. Accordingly, any remaining CD14+ cells in the final drug product would likely be below the lower limit of quantitation (LLOQ) of 0.8%.

In the case where a low remaining level of CD14+ cells internalized the replication incompetent vector, the replication incompetent vector would not amplify upon subsequent secretion because it is replication deficient. Therefore the risk of release into the environment for any potential low level replication incompetent vector by patient shedding is low. In addition, in the event that a low level of replication incompetent vector was shed from the patient into the environment, it would degrade and not propagate in the environment.

Scenarios that can lead to formation of RCL

Formation of RCL during vector manufacture

RCL particles could theoretically be formed during vector production through recombination of viral sequences from the 4 plasmids. Several safety features in the design of the lentiviral system used in replication incompetent vector production are implemented to prevent this risk.

The risk is minimized during the production of the replication incompetent lentiviral vector by using a split-genome third-generation system where the plasmids encoding the segments and genes required to form the replication incompetent viral vector are segregated onto separate plasmids: the envelope glycoprotein (not derived from a lentivirus) is on one plasmid, the *gag* and *pol* genes on another plasmid (derived from HIV-1), the *rev* gene on another plasmid (derived from HIV-1) and the transfer genome encoding the transgene on a separate plasmid (derived from HIV-1 but self-inactivating due to a deletion in the 3'LTR). These sequences are provided *in trans* via transfection of plasmids into the HEK-293T cell line which allows for only transient expression of these constructs during the replication incompetent viral vector production stage.

The risk for RCL is even further reduced by retaining the Rev-dependence of the replication incompetent viral vector: Rev is required for export of the RNA genome transgene from the nucleus into the cytoplasm for protein expression and packaging. Since Rev is provided only *in trans* and since the Rev protein is not packaged in the replication incompetent virus the chance that a lentiviral RNA genome can continue its nuclear export in transduced cells is highly unlikely (see section A2.9).

Finally, the self-inactivating nature of the replication incompetent vector means that expression off of the LTR is significantly reduced due to the 3'LTR deletion and the absence of the HIV-1 *tat* gene (normally required for LTR-driven transcription). Following reverse transcription and integration into the target T cells, the partial *gag* sequence does not contain an active LTR promoter to drive its transcription due to the self-inactivating (SIN) design of the replication incompetent viral vector. (see section A2.9).

To date, there have been no reports of RCL generated during replication incompetent lentiviral vector manufacturing, which may be due, at least in part, to the use of self-inactivating vectors such as the replication incompetent lentiviral vector used in the production of JCAR017.

Although the generation of RCL is unlikely, testing is performed for the presence of RCL at multiple locations in the manufacturing process, beginning with testing of the replication incompetent vector, followed by testing of the final gene-modified T cell product, and finally with subject monitoring after cell infusion.

Formation of RCL due to recombination with retrovirus

Theoretically, formation of a RCL could occur through recombination of the integrated replication incompetent vector with a retrovirus infection in the transduced cells. The inclusion/exclusion criteria provide strict standards that exclude retroviral infections (i.e., HIV, HTLV, HBV, and HCV). Treated subjects are not likely to become infected with HIV post-administration due to general precautionary measures. However, even if this would

occur, homologous sequences need to be present in the T cells and in the viral sequences for recombination to occur. Deletions and changes of viral sequences in replication-deficient lentiviral vector, specifically the LTRs, have caused a reduction of sequences homologous to wild type HIV that do not allow for homologous recombination anymore. Therefore, homologous recombination between the replication-deficient lentiviral vector provirus inserted in the JCAR017 and HIV is not expected. The likelihood of formation of RCL through recombination with a retrovirus is therefore considered negligible.

Formation of RCL through mobilization of human endogenous retroviral sequences (HERVs)

Reversion of replication incompetent vector to replication competency theoretically might also occur as a consequence of recombination between the replication incompetent vector and HERV sequences present in the human genome (Brodsky 1993, Leib-Mosch 1990, Lower 1981, Seifarth 2005, Wilkinson 1993). HERVs are retrotransposons consisting of retrovirus-like DNA sequences such as LTRs and *gag-pol* and *env* open reading frames (ORFs). HERVs entered the human genome as exogenous retroviruses which later became endogenised. HERVs account for 8% of the human genome (Lander 2001) but all known HERVs are replication incompetent due to the accumulation of inactivating mutations and deletions over hundreds of thousands or millions of years. The most complete HERVs belong to the HERV-K group and are present as full length proviruses in the human genome (Turner 2001). The majority of HERVs are related to beta-retroviruses but sequences related to gamma-retroviruses have also been identified by sequence homology searches (Dewannieux 2013, and Villesen 2004).

Sequence homology would be necessary between the replication incompetent vector and HERVs for recombination to occur. The replication incompetent vector only retains a limited portion of lentiviral genome, the LTRs and the extended ψ packaging signal. This limited portion of lentiviral sequences make recombination with HERVs very unlikely. And since all known HERVs are replication incompetent due to inactivating mutations and deletions as described above, even if a recombination event would occur this is unlikely to create a replication-complement virus.

Environmental risk assessment on possible recombination and/or complementation of viral sequences in the lentiviral vector with wild-type viruses in human packaging cells and/or in the patient

The annotated sequence for the ZRX-014-LV replication incompetent vector is proprietary confidential information. Such information is provided in a separate Appendix marked "confidential". Publishing the abovementioned information would disclose confidential, trade secret information and give an advantage to other companies developing similar treatments. The sequence for the replication incompetent viral vector has been provided in this separate Appendix.

The replication incompetent viral vector genome contains sequences that are the minimal *cis* components required for the replication incompetent viral vector genome to complete its intended life cycle of single round transduction. These minimal sequences are described in detail, as well as their origin, in the Appendix. They contain sequences from HIV-1 that are based on the 5'LTR, the psi packaging element which includes a partial *gag* sequence, the Rev-responsive element, the FLAP region, and a partial 3'LTR. These HIV-1 sequences are typical in third generation replication incompetent lentiviral vectors such as ZRX-014-LV. In

addition to HIV-1, there are sequences from Human T cell Leukemia Virus (R element), Woodchuck Hepatitis Virus (WPRE element) and Thosea Asigna virus (T2A peptide-encoding sequence) that serve as regulatory sequences that affect RNA stability, gene expression and protein processing of the transgene.

Concerns regarding homology between vector and HERVs have been addressed above. There are no homologies with the WPRE element or of the T2A sequence with sequences of the human genome and of human pathogens. Therefore recombination is not likely. The HTLV-derived sequence may overlap with HTLV sequences in a patient infected with HTLV. While the overlap could cause a recombination event with HTLV sequences in a patient infected with HTLV, there is low likelihood that an event would occur that could create a scenario where replication competent genetically modified lentiviral vector could occur and disperse into the environment. Regarding homologies between replication incompetent viral vector sequences and the 293T packaging cell line, because this is a human cell line we would expect the same homologies with possible HERV sequences present in the packaging cell line. Since all known HERVs are replication incompetent due to inactivating mutations and deletions as described above, even if a recombination event would occur this is unlikely to create a replication-complement virus. 293T cells have been immortalized using Adenoviral genome. There are no Adenovirus components in ZRX-014-LV.

A5.4. Give an estimate of the chance that the adverse effects described in A5.2 could actually occur.

Give a reasoned estimate of the chance (likelihood) of the aspects described in A5.2 and A5.3, also taking account of the number of test subjects and the dosage.

Likelihood of adverse effects related to the JCAR017 cell product (genetically modified T cells)

The likelihood that adverse effects will occur with JCAR017 cells is very low because the autologous JCAR017 cells are not pathogenic and do not survive outside the subject, as described in A5.2 and A5.3.

Only if accidentally injected into immunocompromised individual, is there a theoretical risk that the JCAR017 cells could persist. The likelihood of this is considered very low. In this case, adverse effects of JCAR017 cannot completely be ruled out. However, the number of JCAR017 cells injected would be much lower than in subjects, which further reduces the likelihood of adverse effects.

Likelihood of adverse effects due to formation of RCL

As described in A5.3, the risk of formation of RCL during either replication incompetent vector production or through recombination in subject cells is negligible. Therefore, the likelihood of adverse effects related to RCL formation is also negligible.

Likelihood of adverse effects related to free replication incompetent **vector particles**

As described in A5.2 and A5.3, the amount of replication incompetent vector particles is JCAR017 cell product is considered to be low and minimal replication incompetent vector can persist in the subject. Therefore, the likelihood of free replication incompetent vector particles spreading to the environment or other individuals in very low.

In the highly unlikely case of accidental injection of JCAR017 cell product containing a very low number of free replication incompetent vector particles into unintended human recipients, the replication incompetent viral particles would likely be inactivated by the complement in human serum. Even if a replication incompetent infectious particle would survive long enough to infect a cell, the vector is replication-deficient and could only integrate once and further spreading is not possible. The likelihood of adverse effects due to a single replication incompetent vector integration is considered extremely low.

A5.5. Describe the risks that could occur as a consequence of the application of the GMO, taking into account the impact of any risk management measures taken.

Describe the risks in such a way that makes clear how the risks can be reduced through risk management. If risk management measures are necessary in order to limit the risks, these should be specified further in the questions below.

The habitat of the JCAR017 cells is restricted to the patient from which the cells were derived. JCAR017 does not have the capacity to disseminate vertically or horizontally in the ecosystem. Accidental injury of the patient and/or loss of blood or blood testing will not lead to dissemination of JCAR017 into the environment, as cells will undergo rapid inactivation in the environment outside the patient.

Dissemination of JCAR017 cells could therefore only occur between human beings. However, persons, other than the patients from whom the cells have been derived, would eliminate the cells as a results of their immune response.

Even though JCAR017 has not been associated with disease, is not pathogenic and does not survive outside its host, exposure of people other than the patients will be avoided. Patients will be instructed not to donate blood, organs, tissues and cells for transplantation for at least 12 months post CAR T infusion and thereafter as long as CAR T cells are detected by qPCR in the peripheral blood. In addition, patients with this disease history are in general not candidates for donation for their lifetime.

Personnel who participate in the administration of blood components must be trained in transfusion procedures, in recognition and management of adverse reactions in the patients and safe handling of genetically modified organisms.

Following administration of JCAR017 cells at the study site, materials used during infusion (e.g., gloves, gauze) will be disposed of in accordance with the institution requirements for GMO waste (Appendix ‘Registratie ggo-afval’).

Only in immunocompromised individuals is there a theoretical risk that the JCAR017 cells could persist and give rise to adverse effects similar to the possible adverse effects in patients (see A2.16). Thus, immunocompromised health care professional should be advised of the risk prior to engaging in work with JCAR017 cells.

Double packaging for internal transport and safe administration procedures are supposed to prevent accidental spills of JCAR017 cell product. However, if a spill occurs despite of these conditions, the spill will be treated with 70% ethanol of 1000 ppm chlorine solution. Following these procedures, accidental release into the environment can be excluded.

Accidental loss of JCAR017 cell product is unlikely to occur due to controlled procedure of transportation, delivery and use, including all necessary documentation.

The amount of free replication incompetent vector particles in the JCAR017 cell product is expected to be negligible (see A3.2). After administration to the patient, any potential remaining replication incompetent viral particles would be inactivated by the complement component of human serum. Therefore, the replication incompetent vector cannot be transmitted to the environment or other individuals from the patient. Theoretically, if a low number of free replication incompetent vector particles were present in the JCAR017 cell product, they could enter a medical health care professional by accidental injection, however this cannot lead to further spreading and is not expected to cause adverse effects (see A5.4).

As described in A5.2, the risk of formation of RCL is negligible. As part of the patient monitoring, patients are tested for RCL. In the very unlikely event of RCL detection, the following mitigation will be implemented:

i. Intensive follow-up of the patient in consultation with gene therapy experts, study investigators, and Health Authorities;
ii. Inform local and country specific public health officials;
iii. Identify sexual partners and provide appropriate counseling and intervention.

In conclusion, the risks to the environment related to the GMO (JCAR017 cell product), and the possibility of free replication incompetent vector particles and/or RCL formation are negligible.

Risk management

A5.6. Which inclusion and exclusion criteria are adopted in the selection of test subjects and what is the effect of these criteria on environmental safety?

Give an overview of the inclusion and exclusion criteria that are <u>only</u> necessary for the protection of the environment or which criteria might possibly have an effect on safety for human beings and the environment.

For recombination of the engineered replication incompetent lentiviral vector with human retroviruses to occur, homologous sequences need to be present in the T cells and in the viral sequences. To date, no RCL has been documented with this type of products. Nevertheless, to eliminate theoretical risk of recombination towards RCL with pathogenic potential, patients with (retroviral) infections (HIV, HTLV, HCV and HBV) are excluded from participating in the clinical studies with JCAR017.

Subjects must agree to not donate blood, organs, sperm or semen, and egg cells for usage in other individuals until at least 12 months after the JCAR017 infusion and until CAR T cells are no longer present by quantitative polymerase chain reaction (qPCR) on two consecutive tests, whichever occurs last.

<u>qPCR transduced T cell assay</u>

The assay is designed for the quantitative determination of JCAR017 replication incompetent vector specific WPRE primer using a quantitative polymerase chain reaction (q-PCR)

assay. The adequacy of the amount of test sample material analyzed is assessed by a second probe and primer set for human albumin (ALB) gene sequences. Samples of genomic DNA (gDNA) isolated from peripheral blood are provided.

This assay has been validated for range of response, intra- and inter-assay accuracy and precision, specificity and selectivity and LOD. As a result of this validation, the following assay parameters and limitations were established:

- Lower limit of quantitation is 25 copies/reaction (125 copies/µg)
- Upper limit of quantitation is 1 x 10^7 copies/reaction (5 x 10^7 copies/µg)
- The Level of Detection is 2.5 copies/reaction (12.5 copies/µg)

Information regarding the qualification of this assay is proprietary confidential information. Such information is provided in a separate Appendix marked "confidential". Publishing the abovementioned information would disclose confidential, trade secret information and give an advantage to other companies developing similar treatments.

A5.7. Describe which measures are provided for in respect of the hospitalization of the test subject.

When answering this question, please emphasize those aspects that are important in preventing spread in the environment of the test subject. Also indicate if, apart from medical reasons, hospitalization is prescribed as a way of protecting against possible effects for humans and the environment.

The primary safety profile of JCAR017 has been established in study TRANSCEND NHL001 which is ongoing in the US. No unexpected early or late toxicities were observed. Major safety findings include cytokine release syndrome (CRS), neurotoxicity (NT) and hematological disorders, including neutropenia, thrombocytopenia, and anemia. No fatal outcome from CRS or NT was reported (Abramson 2017).Studies using JCAR017 in Europe will use products generated with manufacturing steps in Europe. The first study to use this product will be study JCAR017-BCM-001.

For safety reasons, the first 10 patients in study JCAR017-BCM-001treated with JCAR017 will be hospitalized for a minimum of 14 days to assess the early safety profile of the product and to compare it with the safety profile of JCAR017 manufactured solely in the US. After that, hospitalization is at the discretion of the treating physician and based on safety considerations.

No specific human or environmental precautions are required once the patient is discharged from hospital and standard measurements will apply while inpatient.

A5.8. If the test subject is to be hospitalized, what are the criteria for his or her release from hospital?

Describe the criteria on the basis of which the test subject will be released from hospital.

Patients who require hospitalization should not be discharged from the hospital. Patients should remain as inpatients until afebrile for 24 hours and other signs and symptoms of CRS (other than cytopenias) or neurotoxicity reach grade 1 or less and discharge is deemed appropriate in the judgement of the investigator.

A5.9. Describe which measures will be taken to prevent the spread of the GMO to third parties (including medical personnel).

For example, give an overview of relevant (hospital hygiene) measures that will be taken. In case existing guidelines will be used, please indicate what they are (such guidelines must be attached to this application). Additional or deviating measures also must be described.

JCAR017 IMP will be shipped from the GMP manufacturing facility to the clinical site in a validated shipping container according to annex 1 of the GMO regulation. JCAR017 IMP will be thawed on site and administered to the patient via intravenous infusion in a hospital infusion area. The clinical site personnel will be trained in handling and administration, thawing and product accountability procedures. The administration of JCAR017 will be performed at specialized medical centers equipped for the safe administration of biological or cellular products, and by experienced health care professionals, appropriately trained in hygiene procedures and standards regarding safety and infectious materials handling. JCAR017 contains autologous human T cells and therefore, healthcare professionals should employ universal precautions for the prevention of transmission of blood-borne infections as outlined by the Center for Disease Control and Prevention (CDC 1988, Siegel et al., 2007; page 4). The personnel handling the product vials containing JCAR017 will follow the appropriate gowning procedure for cell therapy wearing disposable gloves and an apron. No extra safety measures will be taken for JCAR017. 70% Ethanol will be used for regular disinfection on used surfaces. In case of a spill, the surface will be treated with 1000 ppm Chloride.
Any partially used or unused JCAR017 IMP (material remaining in the vials), the vials, the absorbent barrier pads, any supplies used in the preparation and administration process, including the IV administration set, that contain the GMO or have been in contact with the GMO, must be disposed of in accordance with the GMO regulations (Appendix 'Registratie ggo-afval'). The waste disposal will be registered in the investigator site files.

Procedure in case of unexpected situations and serious incidences

A5.10. Describe the procedures to be followed if changes in the risk management are required for medical reasons.

This may concern situations in which a test subject needs to be removed from isolation; for example because he or she requires intensive care, or in situations when unexpected effects are being observed, including Suspected Unexpected Serious Adverse Reactions (SUSARS) or Serious Adverse Events (SAEs). Also consider the situation of a test subject having died and an autopsy being required.

All unexpected situations and serious incidences must be reported immediately. See the 'Procedure for unwanted incidences' (www.loketgentherapie.nl)

The patient is housed in a standard patient room and JCAR017 is not shed from the treated patient. No additional procedures are required.

A5.11. Describe what aftercare will be given if a test subject ends his/her participation in the study prematurely.

Also state to what extent the aftercare deviates from the aftercare for test subjects who have completed the entire study.

Patients have the right to withdraw from the study at any time and for any reason without prejudice to their future medical care by the physician or at the institution. If a patient ends his/her participation in the study prematurely, the monitoring for up to 15 years will be maintained. If a patient is lost to follow-up after receiving JCAR017 no specific measures will be applied. If the infusion never takes place the product will be returned to the manufacturer.

Monitoring

A5.12. Describe how the monitoring will be set up to identify any spread of the GMO.

In answering this question, pay attention to the method followed, but also to the period during which any positive result can be expected in connection with the applicable scenario. Also state in this context during which period monitoring will take place.

As described in A5.2 and A5.3, there is no scenario in which the GMO can spread from the test subject into the environment. Human T cells will not survive outside the patient. Also, excretion of replication incompetent vector used to manufacture JCAR017 ("shedding") by the patient is not expected to occur. Therefore, no specific monitoring is necessary regarding spread of the GMO.

A6. Conclusions of the possible environmental effects

Directive 2001/18/EC Annex II under Point D.1 gives a number of aspects that should be used whenever applicable as the basis of the conclusions about the possible environmental effects of the introduction of the GMP into the environment. All these points should be taken into account when drafting the conclusions of the risk analysis.

In line with the environmental risk assessment per Directive 2001/18/EC the following are the conclusions for the proposed use of JCAR017:

1. Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s).

There is no likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s). The use of JCAR017 is limited to specialized medical centers under the supervision of qualified study staff in a setting equipped for the safe administration of biological or cellular or genetically modified products that prevent spread to unintended persons.

2. Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realized under the conditions of the proposed release(s).

There is no selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realized under the conditions of the proposed release(s) is negligible. Once infused the transduced T cells do not possess any selective growth advantage in vivo, their half-life time is similar to or lower than the non-transduced T cells.

3. Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species.

There is no potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species. JCAR017 administration is performed within a clinical setting under aseptic conditions. Transmission to other species is not possible. In the event of accidental transmission of the autologous medicinal product to an allogeneic, non-target human the cells would be cleared by the immune system of the individual.

4. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and non-target organisms (if applicable).

There is no known or predicted potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and non-target organisms. JCAR017 administration is performed within a clinical setting under aseptic conditions. Transmission to other species is not possible. In the event of accidental transmission of the autologous medicinal product to an allogeneic, non-target human the cells would be cleared by the immune system of the individual.

5. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release(s).

There are no known or predicted possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working

with, coming into contact with or in the vicinity of the GMO release(s). JCAR017 administration is performed within a clinical setting under aseptic conditions. In the event of accidental transmission of the autologous medicinal product to an allogeneic, non-target human the cells would be cleared by the immune system of the individual.

6. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed.

There is no possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, as JCAR017 is not intended for animal food or any other veterinary product.

7. Possible immediate and/or delayed effects on biogeochemical processes caused by possible direct or indirect interaction between the GMO and the target and non-target organisms in the vicinity of the GMO introduction(s).

There is no possible immediate and/or delayed effects on biogeochemical processes caused by possible direct or indirect interaction between the GMO and the target and non-target organisms in the vicinity of the GMO introduction(s) as the product JCAR017 does not contain replication competent viral particles and the ex vivo transduced T cells are administered to the patient.

8. Possible change in the current medical practice.

The patients to be enrolled in the clinical trial(s) will be following the sponsor's clinical trial protocols implemented by the investigators at that particular study site.

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