

PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF
GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN
ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

- | | | |
|-----|---|--|
| (a) | Member State of notification | Germany |
| (b) | Notification number | B/DE/16/PEI 2903 |
| (c) | Date of acknowledgement of notification | 17/10/2016 |
| (d) | Title of the project | Single center, open label, phase I study of intracranial injection of NK-92/5.28.z cells in patients with recurrent HER2-positive glioblastoma (CAR2BRAIN) |
| (e) | | |
| (f) | Proposed period of release | From 01/12/2017 until 30/11/2019 |

2. Notifier

Name of institution or company:	Sponsor: Goethe University Frankfurt / Main University Hospital Frankfurt Theodor-Stern-Kai 7 60596 Frankfurt / Main Germany
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3. GMO characterisation

(a) Indicate whether the GMO is a:

- | | | |
|----------------|-----|------------------------------------|
| viroid | (.) | |
| RNA virus | (.) | |
| DNA virus | (.) | |
| bacterium | (.) | |
| fungus | (.) | |
| animal | | |
| - mammals | (x) | (genetically-modified NK-92 cells) |
| - insect | (.) | |
| - fish | (.) | |
| - other animal | (.) | |

specify phylum, class ...

(b) Identity of the GMO (genus and species)

Genus: Human

Species: Homo Sapiens Sapiens

The GMO/IMP consists of human lymphocytes (natural killer cells) in form of NK-92 cells transduced ex vivo with the lentiviral vector PS-5.28.z-W (alternative designation: pHR⁻-SF-5.28.z) containing a synthetic codon- optimized gene FRP5hCD8CD28zeta encoding the ErbB2-specific chimeric antigen receptor 5.28.z for the treatment of HER2-positive brain tumors.

(c) Genetic stability – according to Annex IIIa, II, A(10)

Genetic stability – according to Annex IIIa, II, A(10)

A self-inactivating recombinant lentiviral vector (LV) derived from HIV-1 has been chosen to exploit some advantageous properties of lentiviruses: the resulting LV are genetically stable, permanently integrate into the genome of transduced cells and provide long-term gene expression in vitro and in vivo. The NK-92/5.28.z cells display stable CAR expression upon prolonged culture as determined by FACS analysis. It has been shown, that the expression of anti-ErbB2 CAR were stable over more than 9 month in continuous cultures generated from the master cell stock. The staining has been performed by validated means using fluorescent labeled recombinant ErbB2-protein.

The transduction of NK-92 cell line with such LV has been performed under GMP conditions by EUFETS GmbH Idar Oberstein in 2010. Since then, one individual cell clone has been isolated by subcloning and has been rigorously characterized with regard to the absence of shedding and viral safety. This clone, named NK-92/5.28.z has been stored as a master cell bank from which samples will be thawed for clinical evaluation in the respective phase I clinical investigation “CAR2BRAIN”. For detailed vector sequences and classification, please see Annex III. The cell based therapy in the context of the CAR2BRAIN trial, which will test the safety of NK-92/5.28.z (Her2.taNK), does not involve any further gene transduction. In contrast, the well characterized derivative of the natural killer cell line NK-92, which expresses the CAR receptor specific for ErbB2 tumorantigen (NK-92/5.28.z) will be expanded and infused/injected without any further genetic manipulation.

The identity and sequencing of viral batches produced has been tested and verified by sequencing. Absence of release of replication competent viruses has been tested by Bioreliance (Scotland) during the qualification and biosafety level testing of the mastercellbank.

Tests have been performed to verify the identity and integrity of lentiviral vector and transduced cells. The genetic stability of the final Investigational Medicinal Product (i.e.NK-92/5.28.z) was also assessed in order to:

- confirm the presence of the vector integration (assessment of vector copy number),
- confirm the absence of Replication Competent Lentiviruses (RCL), and
- determine the vector’s genomic integration profile.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (.) No (x)

If yes, insert the country code(s) ...

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

	Yes	(.)	No	(x)
If yes:				
-	Member State of notification		...	
-	Notification number		B/././...	

Please use the following country codes:

Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

	Yes	(.)	No	(x)
If yes:				
-	Member State of notification		...	
-	Notification number		B/././...	

7. Summary of the potential environmental impact of the release of the GMOs.

Genetically modified NK-92 cells can only survive ex-vivo under special cell culture conditions in a CO2 incubator at 37°C for a limited period of time, since NK-92 /5.28.z is an IL-2 dependant growing ☐ - irradiated IMP. Thus the environmental risk conferred by inappropriate disposal of waste or unused product or the accidental dissemination during product handling is considered to be negligible. Also, an excretion of live product or its progeny ("shedding") by the patient is unlikely for several reasons: (a) the vector used had a SIN configuration limiting the possibility for replication (b) NK-92 cells were transduced *ex vivo* with infectious particles in 2010 by a contract laboratory under GMP conditions and (c) the cultured cells were negative in tests for the presence of infectious particles therefore passive administration of infectious particles to the patient can be excluded. The risk of passive transfer of vector sequences such as VSVg to the host is negligible based on QC tests of the self-inactivating lentiviral vector. The GTMP will be prepared at the DRK-Blood Donation Service Center in Frankfurt and transported to Frankfurt University Hospital by a dedicated carrier in a sealed S1 container. The actual release will occur within a closed area with restricted personnel access. The staff involved in the release of the GMO has wide experience in GBM treatment and will follow Good Clinical Practice rules.

The GMO is only intended for clinical use and will be administrated immediately after preparation by experienced personnel. Personnel responsible for the administration of the GMO will wear disposable gloves and masks. Standard rules for spraying, wiping, gowning up and entry of personnel/goods into the room are followed. Waste will be collected by trained staff in containers labeled "S1 waste" Waste will be inactivated by autoclaving.

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a:

(select one only)

viroid (.)

RNA virus	(x)
DNA virus	(.)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)
(specify phylum, class) ...	

other, specify human lymphocyte cell line NK-92...

2. Name
- | | | |
|-------|---|------------------------------------|
| (i) | order and/or higher taxon (for animals) | Retroviridae |
| (ii) | genus | Lentivirus |
| (iii) | species | Human immunodeficiency virus (HIV) |
| (iv) | subspecies | HIV-1 |
| (v) | strain | ... |
| (vi) | pathovar (biotype, ecotype, race, etc.) | ... |
| (vii) | common name | Lentiviral vectors |

3. Geographical distribution of the organism

- (a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (.) No (x) Not known (.)

- (b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (x)

If yes, indicate the type of ecosystem in which it is found:

Atlantic	..
Mediterranean	..
Boreal	..
Alpine	..
Continental	..
Macaronesian	..

- | | | |
|-------|-----------|-----|
| (ii) | No | (.) |
| (iii) | Not known | (.) |

- (c) Is it frequently used in the country where the notification is made?
Yes (.) No (x)

- (d) Is it frequently kept in the country where the notification is made?
Yes (.) No (x)

4. Natural habitat of the organism

- (a) If the organism is a microorganism

water (.)
soil, free-living (.)
soil in association with plant-root systems (.)
in association with plant leaf/stem systems (.)
other, specify ...

- (b) If the organism is an animal: natural habitat or usual agroecosystem:
Not applicable.

5. (a) Detection techniques

NK-92/5.28.z cells can be traced in blood, cerebrospinal fluid and other tissues or body fluids by CAR-specific quantitative PCR. In female patients, NK-92/5.28.z cells can also be traced by a y-chromosome specific quantitative PCR since NK-92/5.28.z cells were initially established from a male human subject and contain an endogenous human y-chromosome. As these target sequences are not existent in the patients' own cells, these quantitative PCRs are highly specific. Using these techniques, NK-92/5.28.z cells can reliably be detected up to cell dilutions of $1:1 \times 10^5$.

- (b) Identification techniques

Recipient organism

A sample of the drug substance is collected at the end of the manufacturing process in order to perform a number of Quality Controls (QC). In particular, a transduction efficiency analysis is conducted by measuring the vector copy number integrated, by q-PCR.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

Yes (x) No (.)

If yes, specify

Biosafety level S1

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (.) No (x) Not known (.)

If yes:

- (a) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other (.)

..., B/././...

C. Information relating to the genetic modification

1. Type of the genetic modification

- (i) insertion of genetic material (x)
- (ii) deletion of genetic material (.)
- (iii) base substitution (.)
- (iv) cell fusion (.)
- (v) others, specify ...

2. Intended outcome of the genetic modification

Insertion of a synthetic codon- optimized gene FRP5hCD8CD28zeta in NK-92 cells. The expected result is the expression of a chimeric antigen receptor with specificity to HER-2 positive tumors in natural killer cells of human origin. The CAR will direct the immune reaction of the NK cells towards the tumor.

3. (a) Has a vector been used in the process of modification?
Yes (x) No (.)

If no, go straight to question 5.

- (b) If yes, is the vector wholly or partially present in the modified organism?
Yes (x) No (.)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

- plasmid (.)
- bacteriophage (.)
- virus (x)
- cosmid (.)
- transposable element (.)
- other, specify Recombinant lentiviral vector

(b) Identity of the vector

Lentiviral vector, replication incompetent, designated pHR`-SF-5.28z -
The vector is derived of recombinant RNA.

(c) Host range of the vector

VSV -G (pseudotyped), amphotroph

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

- Yes (x) No (.)

antibiotic resistance (.)

other, specify Flow cytometric determination of integrated FRP5 (CAR) fragment

Indication of which antibiotic resistance gene is inserted
Not applicable

- (e) Constituent fragments of the vector
 Provirus includes a promoter designed to regulate the transgene in NK-92 cells and a transgene designated
- (f) Method for introducing the vector into the recipient organism
- (i) transformation (.)
 - (ii) electroporation (.)
 - (iii) macroinjection (.)
 - (iv) microinjection (.)
 - (v) infection (.)
 - (vi) other, specify *Ex vivo* transduction into NK-92 cell

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

- (i) transformation (.)
- (ii) microinjection (.)
- (iii) microencapsulation (.)
- (iv) macroinjection (.)
- (v) other, specify ...

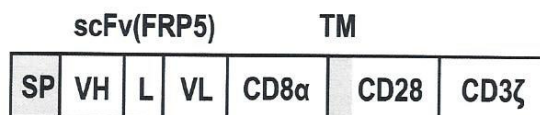
6. Composition of the insert

- (a) Composition of the insert

Composition of CAR 5.28.z

The chimeric antigen receptor (CAR) 5.28.z was assembled as an artificial protein sequence *in silico* from the protein domains indicated below, and then synthesized as a codon-optimized synthetic gene (FRP5hCD8CD28zeta; see below) encoding the protein.

CAR 5.28.z contains the following protein domains (listed from N- to C-terminus):



SP (amino acid positions 1 to 19)

Human immunoglobulin heavy chain signal peptide derived from clone 71-5 (Kodaira et al., 1986); allows expression of the CAR on the cell surface.

scFv(FRP5) (amino acid positions 20 to 260)

Single chain antibody scFv(FRP5) (Wels et al., 1992) consisting of VH, L, VL; enables binding of CAR to the extracellular domain of human ErbB2 protein.

VH (amino acid positions 20 to 138)

Variable region of immunoglobulin heavy chain of murine ErbB2-specific antibody FRP5 (Harwerth et al., 1992; Wels et al., 1992); original amino acid residue Met₁₀₂ (position number according to (Wels et al., 1992)) replaced by Ser.

L (amino acid positions 139 to 153)

Synthetic 15 amino acids linker sequence (Gly₄Ser)₃ stabilizing scFv(FRP5) by connecting variable regions of immunoglobulin heavy and light chains of murine ErbB2-specific antibody FRP5 (Wels et al., 1992).

VL (amino acid positions 154 to 260)

Variable region of immunoglobulin kappa light chain of murine ErbB2-specific antibody FRP5 (Harwerth et al., 1992; Wels et al., 1992).

CD8α (amino acid positions 261 to 322)

Fragment derived from hinge region of human CD8α chain, amino acid residues 117 to 178 of UniProt P01732 isoform 1 (www.uniprot.org/uniprot/P01732); original amino acid residue Cys₁₆₄ (position number according to UniProt P01732) replaced by Ser to prevent formation of unproductive intra- and intermolecular disulfide bonds; provides flexibility to CAR.

CD28 (amino acid positions 323 to 392)

Transmembrane (TM) and intracellular domains of human CD28 costimulatory receptor, amino acid residues 151 to 220 of UniProt P10747 isoform 1 (www.uniprot.org/uniprot/P10747); provides signaling capability to the CAR.

CD3ζ (amino acid positions 393 to 504)

Intracellular domain of human CD3ζ chain (CD247), amino acid residues 52 to 163 of UniProt P20963 isoform 3 (www.uniprot.org/uniprot/P20963); provides signaling capability to the CAR.

- (b) Source of each constituent part of the insert

See (a)

- (c) Intended function of each constituent part of the insert in the GMO

See (a)

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (x)
- other, specify ...

(e) Does the insert contain parts whose product or function are not known?

Yes (.) No (x)

If yes, specify ...

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

viroid (.)

RNA virus (.)

DNA virus (.)

bacterium (.)

fungus (.)

animal

- mammals (.)

- insect (.)

- fish (.)

- other animal (.)

(specify phylum, class) ...

other, specify The donor is the self-inactivating pHR⁺-5.28.z lentiviral vector containing the codon-optimized synthetic gene FRPhCD8CD28 zeta

2. Complete name

not applicable

(i) order and/or higher taxon (for animals) ...

(ii) family name for plants ...

(iii) genus ...

(iv) species ...

(v) subspecies ...

(vi) strain ...

(vii) cultivar/breeding line ...

(viii) pathovar ...

(ix) common name ...

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (.) No (x) Not known (.)

If yes, specify the following:

(b) to which of the following organisms:

humans (.)
 animals (.)
 plants (.)
 other ..

- (b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
 Yes (.) No (x) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):
 ...

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
 Yes (x) No (.)

If yes, specify The lentiviral vector used to transduce the human cell line NK-92 is classified as group 2, class 2 and containment L2. The plasmids which were used to generate the replication incompetent and pseudotyped vector are classified as biosafety level 1. (5.Do the donor and recipient organism exchange genetic material naturally?
 Yes (.) No (x) Not known (.)

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

- (a) is the GMO different from the recipient as far as survivability is concerned?
 Yes (.) No (x) Not known (.)

Specify The GMO consists of genetically modified NK-92 cells. No influence on cell survival is expected from the vector or the insert contained in the vector. The only phenotypic modification is the expression of the transgene by the genetically engineered NK-92 cells.

- (b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
 Yes (.) No (x) Unknown (.)

Specify No influence on reproduction is expected from the vector or the insert contained in the vector.

- (c) is the GMO in any way different from the recipient as far as dissemination is concerned?
 Yes (.) No (x) Not known (.)

Specify The GMO is derived from NK-92 cells. This cell line can only survive under specific laboratory conditions and, furthermore maintenance of cells is dependent on the supplementation of the cell culture medium with IL-2.

- (d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
 Yes (.) No (x) Not known (.)
 Specify The genetic modification of NK-92 will not result in any changes in the pathogenicity of the genetically modified cells for the environment, as determined by the experimental assays currently available. No RCL release was observed by analyzing the supernatant of the genetically-engineered NK-92 cells.

2. Genetic stability of the genetically modified organism

The insert is stably integrated into the genome of the cell, and does not have the capacity for mobilization. The pHR'-SF- 5.28.z lentiviral vector is stable and does not have capacity for replication.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes (.) No (x) Unknown (.)

(a) to which of the following organisms?

humans (.)
 animals (.)
 plants (.)
 other ...

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

Genetically modified NK-92 cells can only survive ex-vivo under special cell culture conditions in a CO2 incubator at 37°C. Outside of the incubator the GMOs are not viable. Thus the environmental risk conferred by inappropriate disposal of waste or unused product or the accidental dissemination during product handling is considered to be negligible. Also an excretion of live product or its progeny ("shedding") by the patient is unlikely for several reasons: (a) the vector has a SIN configuration limiting the possibility for replication (b) NK-92 cells are transduced ex vivo with infectious particles and (b) the cultured cells are tested for the release of RCL and no RCL were observed. (c) cells will be gamma-irradiated before intracranial administration into the patient and cells do not engraft or proliferate. The risk of passive transfer of vector sequences such as VSVg to the host is very low based on QC tests of the lentiviral vector showing undetectable transfer of viral sequences to a permissive indicator cell line following infection with the vector.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

The final GMO is not stable under uncontrolled environmental conditions. It is intracranially injected into the patients' brain tumor or involved brain tissue. The GMO can be identified by means of CAR expression (transgene) in the patients' tissue and blood, which will be monitored by flow cytometry and

immunohistochemistry. Detection of the GMO in the environment (for example after a spill) is possible using PCR methods that detect the unique sequence.

- (b) Techniques used to identify the GMO
The GMO can be identified by counting vector copy number (Method: q-PCR for vector-specific sequences normalized to housekeeping genes).

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
The GTMP is not released in the environment other than intracranial injection to patients enrolled in this phase I clinical trial with recurrent HER-2 positive brain tumors. Selective and restrictive effecting of NK-92/5.28.z by means of cytotoxic activity against ErbB2-positive tumor cells is aimed.
2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?
Yes (.) No (X.)
If yes, specify NK-92 cells are natural killer cells which are derived from a human donor.
3. Information concerning the release and the surrounding area
 - (a) Geographical location (administrative region and where appropriate grid reference):
The IMP is administered to the patient at the clinical trial site (operating room) in Frankfurt, Germany:

Center for Neurology and Neurosurgery
University Hospital Frankfurt
Frankfurt / Main, Germany
 - (b) Size of the site (m²): 20 m²
 - (i) actual release site (m²): ... m²
 - (ii) wider release site (m²): ... m²Not applicable, since the GMO will be injected into the brain tissue during surgical intervention. The release area is therefore confined to the cancer patient and will represent cm².
 - (c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not applicable
 - (d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Not applicable
4. Method and amount of release
 - (a) Quantities of GMOs to be released:
The patient will receive a single infusion in a volume of up to 2 ml intracranial according to the dosage scheme

- (b) Duration of the operation:
The final GMO infusion will last several minutes.
- (c) Methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of the release
The GMO will be prepared at the DRK-BSD Frankfurt and transported to the intervention room at the Frankfurt University hospital by trained staff in a sealed S1 container, together with a spill kit and accompanying documents. The actual "release" will occur within a closed area (operation room) with restricted personnel access. The staff involved in the release of the GMO has wide experience in the treatment of brain tumors and will follow Good Clinical Practice rules as in any stem cell transplantation routine.
The GMO is only intended for clinical use and will be administrated immediately after arrival by experienced personnel. Personnel responsible for the administration of the GMO will wear disposable gloves and masks. Standard rules for spraying, wiping, gowning up and entry of personnel/goods into the room are followed. Remaining waste and IMP remnants will be transported in sealed containers labeled with appropriate stickers to be inactivated.

5. Short description of average environmental conditions (weather, temperature, etc.)
Controlled standard conditions of an operation room.
6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.
Not applicable. No data is available for this particular GMO. However the GMO cannot survive outside of the laboratory space.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)
- | | | |
|--------|---|-----|
| (i) | order and/or higher taxon (for animals) | ... |
| (ii) | family name for plants | ... |
| (iii) | genus | ... |
| (iv) | species | ... |
| (v) | subspecies | ... |
| (vi) | strain | ... |
| (vii) | cultivar/breeding line | ... |
| (viii) | pathovar | ... |
| (ix) | common name | ... |
2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
Anticipated mechanism of interaction is the antitumor effect of the GMO in patients with advanced brain tumors.
...
3. Any other potentially significant interactions with other organisms in the environment

Interactions with other organisms in the environment are not possible due to the nature of the GMO (human cell line)

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes (.) No (x) Not known (.)

Give details

As indicated, the GMO represents a human cell line which is only capable of limited survival in a human host. Moreover the GMO will be gamma-irradiated before administration to the patient and therefore not mitotically active. Irradiated NK-92/5.28.z cells die within 72 hours within the human patient. Outside of controlled cell culture conditions and outside of the host survival and invasion is not possible.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

None

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

- | | | |
|--------|---|-----|
| (i) | order and/or higher taxon (for animals) | ... |
| (ii) | family name for plants | ... |
| (iii) | genus | ... |
| (iv) | species | ... |
| (v) | subspecies | ... |
| (vi) | strain | ... |
| (vii) | cultivar/breeding line | ... |
| (viii) | pathovar | ... |
| (ix) | common name | ... |

None- not applicable

7. Likelihood of genetic exchange in vivo

- (a) from the GMO to other organisms in the release ecosystem:
Not possible

- (b) from other organisms to the GMO:
Not possible

- (c) likely consequences of gene transfer:
none ...

8. Give references to relevant results (if available) from studies of the behavior and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
similar GMO's, representing CAR T lymphocytes (in contrast to CAR natural killer cells), have been clinically used in many patients with advanced tumors. No ecological impact has been observed in any patient treatment.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
None

H. Information relating to monitoring

1. Methods for monitoring the GMOs
The GMO can be monitored by nucleic acid amplification (PCR) due to the specific sequence of the chimeric antigen receptor.
2. Methods for monitoring ecosystem effects
The “release” of the GMO will be representing the local injection in to the tumor of patients and is expected to have a therapeutic effect throughout the limited life span of the GMO in the patient (up to 72hrs). Environmental monitoring will be done occasional by wiping surfaces and performing a GMO specific PCR in the case of spills.
3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Not applicable. The surgical intervention room does not host other organisms
4. Size of the monitoring area (m²)
20 m²
Only applicable during infusion of the GMO to the patient (operating room/infusion room).
5. Duration of the monitoring

until the cleaning has been shown effective
6. Frequency of the monitoring
Only upon spill or breakage

I. Information on post-release and waste treatment

1. Post-release treatment of the site
The site/place of the GMO administration will be cleaned and disinfected according to standard cleaning methods for handling of biological hazard materials established in the hospital.
2. Post-release treatment of the GMOs
All IMP waste, as well as any material that came into contact with the IMP will be destroyed according to the hospital facility bio-hazard disposal procedures. The waste is to be deactivated by autoclaving or utilization of a disinfectant prior to incineration.
3. (a) Type and amount of waste generated
Needles, syringes, cotton balls, dry adhesives, gloves, and disposable garments. Sharps (needles etc.) will be stored in different specific containers appropriately labeled. The estimated total amount of waste is expected to be minimal (less than 1000 ml). throughout the clinical trial

3. (b) Treatment of waste
Waste will be collected by trained staff in containers labeled “S1 waste” Waste will be inactivated by autoclaving

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
A transparent sterile sealing bag serves as secondary packaging for the IMP and, following labeling, NK-92/5.28.z is immediately shipped to the clinical trial center in a validated transport box at 22±4 °C together with an accompanying document and a spill kit for the inactivation of the IMP in case of spill or breakage.
2. Methods for removal of the GMO(s) of the areas potentially affected
A spill kit is available for proper disinfection.
3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
See J1
4. Plans for protecting human health and the environment in the event of an undesirable effect
NK-92/5.28.z is only intended for clinical use and will be administrated immediately after preparation by experienced personnel. Personnel responsible for the administration of the IMP will wear disposable gloves and masks. Standard rules for spraying, wiping, gowning up and entry of personnel/goods into the room are followed. Waste will be collected by trained staff in containers labeled “S1 waste” Waste will be inactivated by autoclaving.