EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Zalmoxis

Common name: allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) and the herpes simplex I virus thymidine kinase (HSV-TK Mut2)

Procedure No.: EMEA/H/C/002801/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

AE	Adverse Event
ALL	Acute Lymphoblastic Leukaemia
AML	Acute Myeloid Leukaemia
ATMP	Advanced Therapy Medicinal Product
CI	Confidence Interval
Cmax	Median Peak Count
CMV	Cytomegalovirus
CR	Complete Response
DFS	Disease Free Survival
ΔLNGFR	Truncated form of the human Low affinity Nerve Growth Factor Receptor
DLI	Donor Lymphocytes Infusion
EBMT	European Bone Marrow Transplant
EBV	Epstein-Barr virus
GCP	Good Clinical Practice
G-CSF	Granulocyte-Colony Stimulating Factor
GCV	Ganciclovir
GvHD	Graft versus Host Disease
GvL	Graft versus Leukaemia
HR	Hazard Ratio
HSCT	Haematopoietic Stem Cell Transplant
HSV-TK	Herpes Simplex Thymidine Kinase
IMP	Investigational Medicinal Product
IQR	InterQuartile Range
IR	Immune Reconstitution
ISCT	International Society of Cell Therapy
ITT	Intent To Treat
MAA	Marketing Authorisation Application
NCI-CTC	National Cancer Institute Common Toxicity Criteria
NK	Natural Killer
NRM	Non-relapse mortality
OS	Overall survival
PBMC	Peripheral Blood Mononuclear Cells
PDCO	Paediatric Committee
PFS	Progression Free Survival
PIP	Paediatric Investigation Plan
PS	Performance Score
RAEB(-T)	Refractory anaemia with excess of blasts (in transformation)
RI	Relapse Incidence
RCR	Replication Competent Retrovirus
SD	Standard Deviation
SE	Standard Error
TBI	Total Body Irradiation
Tmax	Median Time to Reach the Peak

1. Background information on the procedure

1.1. Submission of the dossier

The applicant MolMed SpA submitted on 5 March 2014 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Zalmoxis, through the centralised procedure falling within the Article 3(1) 1of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 20 September 2012.

Zalmoxis was designated as an orphan medicinal product EU/3/03/168 on 20 October 2003 in the following condition: adjunctive treatment in hematopoietic cell transplantation.

The applicant applied for the following indication: Zalmoxis is indicated as adjunctive treatment in haploidentical haematopoietic stem cell transplantation of adult patients with high-risk haematological malignancies.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Zalmoxis as an orphan medicinal product in the approved indication. The outcome of the COMP review can be found on the Agency's website: ema.europa.eu/Find medicine/Human medicines/Rare disease designation.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that 'Allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) and the herpes simplex I virus thymidine kinase (HSV-TK Mut2)' was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/057/14 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/057/14 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

Applicant's request for consideration: Conditional Marketing Authorisation

The applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14(7) of Regulation (EC) No 726/2004 based on the following claims:

Zalmoxis falls under the following categories regarding the scope of conditional marketing authorisation as laid down in Article 2 of Regulation (EC) No 507/2006:

- "medicinal products which aim at the treatment, the prevention or the medical diagnosis of

seriously debilitating diseases or life-threatening diseases"

- "medicinal products designated as orphan medicinal products in accordance with Article 3 of Regulation (EC) No 141/2000"

The applicant considers that the requirements for conditional marketing authorisation as laid down in Article 4 of Regulation (EC) No 507/2006 are met:

(a) the risk-benefit balance of the medicinal product, as defined in Article 1(28a) of Directive 2001/83/EC, is positive;

(b) it is likely that the applicant will be in a position to provide the comprehensive clinical data;

(c) unmet medical need will be fulfilled;

(d) the benefit to public health of the immediate availability on the market of the medicinal product concerned outweighs the risk inherent in the fact that additional data are still required.

New active Substance status

The applicant requested the active substance 'Allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) and the herpes simplex I virus thymidine kinase (HSV-TK Mut2)' contained in the above medicinal product to be considered as a new active substance in itself, as the applicant claims that it is not a constituent of a product previously authorised within the Union.

The applicant MolMed SpA submitted on 30 July 2009 an application for scientific recommendation on Classification to the European Medicines Agency (EMA) for Zalmoxis, which was designated as an Advanced Therapy Medicinal Product on 16 October 2009.

Protocol Assistance

The applicant received Protocol Assistance from the CHMP on 7 December 2011, 17 November 2011, 22 October 2009, 7 February 2007 and 19 November 2004. The Protocol Assistance pertained to quality, non-clinical and clinical aspects of the dossier.

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

CAT Rapporteur: Johannes H. Ovelgönne CAT Co-Rapporteur: Sol Ruiz

CHMP Coordinator (Rapporteur): Pieter de Graeff

CHMP Coordinator (Co-Rapporteur): Concepcion Prieto Yerro

- The application was received by the EMA on 5 March 2014.
- The procedure started on 26 March 2014.
- The CAT agreed to consult the national ERA authorities on the Environmental Risk Assessment of the GMO as the ATMP is a somatic cell therapy medicinal product. The consultation procedure closed on 1 April 2016.
- The Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 14 June 2014. The Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP

members on 13 June 2014.

- The PRAC Rapporteur's Risk Management Plan (RMP) Assessment report was endorsed by PRAC on 10 July 2014.
- During the meeting on 18 July 2014, the CAT greed on the consolidated List of Questions to be sent to the applicant.
- During the meeting on 24 July 2014, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 24 July 2014.
- The applicant submitted the responses to the CAT consolidated List of Questions on 27 January 2015.
- GMP and GCP inspections were requested and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CAT and CHMP members on 4 March 2015.
- The PRAC RMP Advice and assessment overview was adopted by PRAC on 12 March 2015.
- During the CAT meeting on 20 March 2015, the CAT agreed on a list of outstanding issues to be addressed in writing by the applicant.
- During the CHMP meeting on 26 March 2015, the CHMP agreed on a list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CAT list of outstanding issues on 20 November 2015.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the list of outstanding issues to all CAT and CHMP members on 5 January 2016.
- The PRAC RMP Advice and assessment overview was adopted by PRAC on 14 January 2016.
- During the CAT meeting on 22 January 2016, the CAT agreed on a second list of outstanding issues to be addressed in writing by the applicant.
- During the CHMP meeting on 28 January 2016, the CHMP agreed on a second list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the second CAT list of outstanding issues on 22 February 2016.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the second list of outstanding issues to all CAT and CHMP members on 11 March 2016.
- The PRAC RMP Advice and assessment overview was adopted by PRAC on 17 March 2016.
- During the CAT meeting on 23 March 2016, the CAT agreed on a third list of outstanding issues to be addressed in writing and in an oral explanation by the applicant.
- During the CHMP meeting on 1 April 2016, the CHMP agreed on a third list of outstanding issues to be addressed in writing and in an oral explanation by the applicant.
- The applicant submitted the responses to the third CAT list of outstanding issues on 20 April 2016.

- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the list of outstanding issues to all CAT and CHMP members on 4 May 2016.
- The PRAC RMP Advice and assessment overview was adopted by PRAC on 13 May 2016.
- During the CAT meeting on 18 May 2016, outstanding issues were addressed by the applicant during an oral explanation.
- The CAT/CHMP agreed on a fourth list of outstanding issues to be addressed in writing and in an oral explanation by the applicant on 30 May 2016.
- The applicant submitted the responses to the fourth CAT/CHMP list of outstanding issues on 31 May 2016.
- The CAT/CHMP adopted a report on similarity of Zalmoxis versus Mozobil and Tepadina on 17 June 2016.
- On 21 June 2016, the CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive scientific opinion to Zalmoxis.
- During the meeting on 23 June 2016, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive scientific opinion to Zalmoxis.

2. Scientific discussion

2.1. Introduction

During haematopoietic stem cell transplantation (HSCT) allogeneic haematopoietic stem cells (HSCs) are derived from a healthy donor and transplanted to a patient. It is regarded as a potentially curative procedure for a variety of malignant and non-malignant conditions. The preferred donor for an HSCT candidate is an HLA-identical sibling. Given dominant inheritance patterns, there is a 25% chance that a given sibling will be HLA-identical with a patient. Overall, approximately 15–30% of patients referred for allogeneic HCT have suitable HLA-identical sibling donors. For patients without sibling donors, a search for an HLA-matched unrelated donor can be undertaken. For patients in whom allogeneic HSCT is indicated, but who lack a suitable HLA-matched unrelated donor, three alternative stem cell sources are potentially available: HLA-mismatched unrelated donors; umbilical cord blood and HLA-haploidentical (family) members.

Each approach brings a set of challenges. In the case of HLA-mismatched unrelated-donor HSCT, Graft versus Host disease (GvHD) remains a major issue, and some protocols have studied more intensive prophylaxis in these patients. Umbilical cord blood has unique properties of immunologic naivety, but brings about issues as the small unit size, slow engraftment and substantial rates of opportunistic infection. To solve these issues, the most promising approaches at present involve the use of double-cord HSCT and/or ex vivo expansion of progenitor cells to augment the cord blood cell dose.

The graft in the haploidentical HSCT setting is typically derived from a parent, sibling or child of the patient. Many patients have such a donor available. Given the profound HLA disparity involved, early attempts at HLA-haploidentical HSCT were unsuccessful, owing to severe and often fatal hyperacute GvHD, as well as immunologic graft rejection. Current approaches to haploidentical HSCT rely on T-cell depletion from the allograft to ameliorate these reactions and overcome the HLA disparity. With advances in positive selection of CD34+ cells, encouraging rates of engraftment and disease control with acceptable rates of GvHD have been reported when transplanting high numbers of CD34+ cells /kg combined with a limited number of T-cells added back to the graft. The Johns Hopkins group has taken an alternate approach to T-cell depletion in HLA-haploidentical HCT, administering the lymphotoxic agent cyclophosphamide in one or two doses several days after stem cell infusion, with the goal of selectively depleting activated alloreactive donor lymphocytes in vivo. *(Gyurkocza et al., Expert Rev Hematol 2010)*

HSCT can be associated with prolonged immunodeficiency post-transplantation, especially after extensive treatment for underlying malignancies and the use of T-cell-depleted grafts. Considerable time (about 1–2 years) is needed for the complete regeneration of the T-cell and B-cell compartments, especially when the thymus has lost most of its function owing to age or prior therapies. GvHD and the immunosuppressive drugs used for its prevention can also severely delay immune reconstitution. During this period, patients are subject to opportunistic infections, which in many cases are fatal. Thus, effective approaches to hastening immune reconstitution following transplantation are needed. *(Wei Li et al., Nature Immunology Reviews 2012)*

Zalmoxis is an Advanced Therapy Medicinal Product based on somatic T-cells genetically modified to express the Herpes Simplex Thymidine Kinase (HSV-TK) suicide gene and a truncated form of the human Low Affinity Nerve Growth Factor Receptor (ΔLNGFR) genes (for identification of transduced cells).

Zalmoxis therapy is intended as adjunctive therapy in patients who underwent haploidentical hematopoietic stem cell transplantation (HSCT) in order to aid immune reconstitution (IR). A hastened IR would subsequently prevent the onset of infectious diseases and thus result in a lower treatment-related or non-relapse mortality (NRM) and fewer disease relapse.

The finished product (FP) proposed for the present application is an ATMP based on somatic T-cells genetically modified to express the HSV-TK suicide gene and ΔLNGFR genes (for identification of the transduced cells). The FP has been classified by the Committee for Advanced Therapies (CAT) as an ATMP, somatic cell therapy medicinal product, as defined in Dir. 2009/120/EC amending Directive 2001/83/EC Annex I part IV (EMEA/CAT/419154/2009).

The HSV-TK suicide gene therapy is based upon the observation that the efficacy of allogeneic HSCT strongly relies on the immune advantage conferred by donor T-cells that are able to mediate a potent graft-versus-leukaemia effect and effectively control the opportunistic infections. The post-transplantation administration of donor T-cells aims to improve immunological reconstitution, to facilitate engraftment, and to mediate long-lasting anti-leukaemia effects. However, the post-transplantation administration of T-cells is also associated with occurrence of GvHD.

Zalmoxis is constituted by donor's T lymphocytes genetically modified to express the HSV-TK gene, as suicide gene. This allows the selective killing of dividing cells upon administration of the pro-drug ganciclovir (GCV), which is enzymatically phosphorylated to an active triphosphate analogue by HSV-TK. Triphosphate GCV competitively inhibits incorporation of deoxyguanosine triphosphate (GTP) into elongating DNA, thus killing the proliferating cells.

If GvHD occurs, ganciclovir/valganciclovir will be administered. The activated, transduced T-cells that are causing the GvHD should convert the GCV to its toxic form and thereafter undergo apoptosis. This strategy should allow the direct targeting of those T-cells that are initiating the GvHD response and should permit to avoid/reduce the administration of the immunosuppressive therapies usually given in the post-transplantation phase to prevent or treat GvHD.

The claimed indication is: *"Zalmoxis is indicated as adjunctive treatment in haploidentical haematopoietic stem cell transplantation of adult patients with high-risk haematological malignancies."* The recommended dose and schedule is $1x10^7$ cells/kg given as intravenous infusion every 30 days for a maximum of four times until a circulating T-cell count higher than 100 per µL.

The medicinal product is administered by intravenous infusion, at the foreseen dose of $1x10^7$ cells/kg. The first administration should occur between day 21 to day 49 after HSCT. In case of failed IR, calculated as CD3+ <100/ µl, up to 3 further infusions were performed at 30 day intervals each.

The Applicant received CHMP scientific advice (Protocol Assistance) on several occasions during the development of the product on regulatory, quality, toxico-pharmacological and clinical aspects and scientific recommendation on classification of ATMP according to Article 17 of Regulation (EC) No. 1394/2007.

2.2. Quality aspects

2.2.1. Introduction

The finished product (FP) is presented as a cell dispersion for infusion; containing $5\text{-}20x10^6$ cells/ml allogeneic T cells genetically modified to express the Herpes Simplex Thymidine Kinase (HSV-TK) suicide gene and a truncated form of the human Low Affinity Nerve Growth Factor Receptor (ΔLNGFR) genes (for identification of transduced cells) as active substance.

Other ingredients are sodium chloride, human serum albumin and dimethyl sulfoxide.

The medicinal product is available as one individual treatment dose in 50-500 mL ethylene-vinyl-acetate cryo bag containing a volume of 10-100 mL of frozen dispersion at the concentration of 5-20 $x10^6$ cells/mL.

2.2.2. Active Substance

General information

Zalmoxis contains allogeneic T cells genetically modified to express a suicide gene (referred to by the Applicant as MM-TK) as active substance. The allogeneic T-cells have been genetically modified to express the Herpes Simplex Thymidine Kinase (HSV-TK) suicide gene and a truncated form of the human Low Affinity Nerve Growth Factor Receptor (ΔLNGFR) genes (which serves for the identification of transduced cells).

Allogeneic T cells are obtained from lymphocyte aphaeresis of haploidentical donors.

Genetic modification is achieved by *ex vivo* transduction with the **SFCMM-3 Mut2 #48 retroviral vector** encoding for a mutated form of the HSV-TK gene and the ΔLNGFR gene.

Manufacture, characterisation and process controls

Manufacture of the active substance takes place at MolMed SpA, Via Olgettina 58, 20132 Milan, Italy. This site is further responsible for the analytical testing and release of the active substance. This site is authorised for the manipulation and handling of GMOs.

In the manufacture of Zalmoxis two biopharmaceutical starting materials are used, the retroviral vector (SFCMM-3 MUT2 #48) and peripheral blood mononuclear cells (PBMCs).

The manufacture of the retroviral vector (SFCMM-3 MUT2 #48) and peripheral blood mononuclear cells (PBMCs) is described below.

- **Manufacture of the SFCMM-3 MUT2 #48 retroviral vector**

The SFCMM-3 Mut2#48 vector is a replication-defective retroviral vector derived from MoMu. The establishment of the SFCMM-3 Mut2 vector has been described in sufficient detail.

Establishment of SFCMM-3 Mut2#48 vector cell banks

A cell bank system made of a Master Cell bank (MCB), a Working Cell Bank (WCB) and a Post Production Cell Bank (PPCB) has been established. Cell banks were tested for identity, functionality and adventitious agents. Upon request, additional characterisation of the cell banks was performed in line with current guidance on cell substrates (ICH/WHO/Ph. Eur.).

Adequate information was provided on the sequence of the provirus in PPCB and on the implications of the genetic instability with respect to vector copy number in MCB and PPCB. As requested, the Applicant has introduced additional testing of the cell

SFCMM-3 Mut2#48 vector manufacture/control of materials

The SFCMM-3 Mut2 #48 retroviral vector supernatant is produced from the WCB, serially split in flasks and in cell factories (CF), satisfactory data on validation (active substance and finished product), stability (vector, active substance and finished product), and comparability (vector, active substance and finished product) have been provided.

SFCMM-3 Mut2#48 vector process development

The TK007 clinical phase I/II studies were performed with material obtained by vector SFCMM-3 #35 . Prior to initiation of TK008 phase III studies, the retroviral vector was re-designed to avoid the generation of spliced forms (SFCMM-3 Mut2 #48). Furthermore, the process was scaled up and improvements to the safety of certain raw materials were made (process B). Process changes have been adequately described and the rationale for the changes is valid.

During the procedure concern was raised in relation to comparability between vectors manufactured according to the different processes, which was considered insufficiently supported by the provided data.

During the procedure new data were provided that demonstrated comparability between vectors

SFCMM-3 Mut2#48 vector process validation

Process validation was developed in a step-wise approach, which mainly consists of the following phases: small scale studies to investigate the cell growth, metabolism and productivity of the cells, and validation of full scale manufacture at the level of cell expansion, bioreactor inoculum and growth phase, production and harvest, filtration and filling. Process parameters and quality attributes of the viral vector bulk were analysed.

The Applicant has provided data from three full scale validation batches manufactured according to the commercial vector manufacturing. *Control of the SFCMM-3 Mut2#48 vector*.

A thorough quality control of each batch of retroviral vector is considered essential in view of the low frequency of production and to ensure viral safety of the finished product, as no viral removal or inactivation steps are included further downstream. The quality attributes tested for are considered adequate for determining the vector identity and properties, potency of the vector and the absence of adventitious agents. With regard to impurities, upon request, the Applicant has added a test for residual host cell proteins to the release test panel.

The description of the analytical methods is sufficient. Upon request, additional information on method validation, including a number of validation reports, have been provided. Several issues related to method validations have been resolved. Some minor issues remain to be addressed, but this can be done post-authorisation.

Stability of the SFCMM-3 Mut2#48 vector

The SFCMM-3 Mut2 #48 retroviral vector stability is evaluated according to a program including real time (≤ -65° C), accelerated (-20° C) and stressed (+5° C) storage conditions at different time points. .

- **Manufacture of peripheral blood lymphocytes (PBMCs)**

Haplo-identical donor peripheral blood lymphocytes are obtained by lymphocyte aphaeresis. Total mononuclear cells, collected in a dedicated bag and transported and processed. Donors are screened in agreement with the directive (2006/17/EC) and for the presence of mycoplasma. Upon request, additional information was provided regarding the presence of EBV screening and the risk of reactivation of latent viruses in the PBMCs.. Final cell suspension of PBMCs is collected and diluted in freezing medium containing HSA 7% and 10% dimethylsulphoxide (DMSO), frozen and stored in liquid nitrogen vapour. During the procedure, the Applicant has provided detailed information on the isolation of PBMCs from the apheresis material and the freezing of these cells. In addition, the Applicant has identified critical process parameters (CPPs) that can impact the critical quality attributes (CQAs) of the isolated PBMCs and has performed a failure mode and effects analysis (FMEA) risk assessment. Data have been provided that support the proposed shelf life of the PBMCs. PBMCs processing and quality control is performed at MolMed. The PBMCs have a specification for viability, sterility, endotoxin.

Zalmoxis active substance (Allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) and the herpes simplex I virus thymidine kinase (HSV-TK Mut2))

Manufacture of active substance

The manufacturing process of Zalmoxis active substance comprises the transduction of stimulated peripheral blood lymphocytes (PBMCs) with retroviral vector (SFCMM-3 Mut2#48 vector) and subsequent cell expansion and selection of transduced cells.

At each stage, a sample is taken for microbial control as in-process control.

Process validation and control of critical steps and intermediates

Since only a change in the formulation medium occurs between Zalmoxis active substance and the finished product, the manufacturing process is considered a single intervention starting from thawing the donor PBMCs to the production of the finished product. Therefore, the validation exercise covers both the active substance and the finished product manufacturing processes.

The manufacturing process has continuously been in development and many changes have been introduced while the clinical studies were on-going. As a result, for process validation and evaluation batch data from different stages were presented as follows: full scale evaluation runs, full scale transfer runs, full-scale GMP runs (Process validation batches), continuous process verification (batches from Phase III clinical study) and validation of the aseptic process. Upon request process validation data have been provided for vector produced according to the commercial vector manufacturing process.

Process consistency was assessed. All batches were in agreement with the specification set.

Understanding of the process parameters for the various steps in the process is limited but sufficient, especially because narrow operating ranges have been set. Validation data have been provided for finished product manufacturing. The applicant provided information on the rationale of the CPPs and their ranges and justified that they can be maintained within the specified ranges during normal operation. Not all process steps have been demonstrated to be optimised. Robustness of the transduction step has been addressed by using a fixed MOI, which yields consistent results.

Cellular impurities have been evaluated. The capability to manufacture aseptically has been appropriately validated.

Manufacturing process development

A number of changes and improvements were made to the manufacturing process throughout clinical development. Between Phase I/II and the Phase III study there was a more substantial change in the manufacturing process. The vector used for transduction was changed from #35 to #48, with a different sequence and manufactured in a different cell line with a different vector manufacturing process.

To demonstrate comparability between the commercial process and previous versions of the processes, the Applicant submitted data on validation (active substance and finished product), stability (vector, active substance and finished product), and comparability (vector, active substance and finished product). These validation data were considered adequate to confirm comparability for vector in a head-to-head comparison. The comparability of viral vector commercial manufacturing process with previous versions of the process was considered to be demonstrated.

Characterisation

The parameters analysed in the characterisation studies include identity, purity, potency and safety.

Focus of the product characterisation was the immunophenotype profile.. The applicant committed to continue the analysis of cellular markers throughout the Phase III clinical study and submit the final analysis of the expression of the cellular markers after finalisation of the phase III clinical study.

Specification

The set of specifications for Allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) and the herpes simplex I virus thymidine kinase (HSV-TK Mut2) are detailed in the dossier. Since only a change in the formulation medium occurs between Zalmoxis active substance and the finished product, the manufacturing process is considered a continuous process from thawing the donor PBMCs to the production of the finished product. Therefore, there is no holding step at the level of the active substance and this is reflected in the limited set of parameters that are tested at the level of the active substance specifications. This was considered acceptable.

Of note, cell number was not included as release specification neither for active substance nor for finished product. The required cell number will be dependent on the patient's weight and the Applicant has provided adequate clarification on how the required cell number is targeted during active substance and finished product manufacture (one individual treatment dose contains a volume of 10-100 mL of frozen dispersion at the concentration of 5-20 x 10^6 cells/mL).

Stability

As the manufacturing process is a continuous process and no holding step for the active substance is introduced no information is included on the active substance stability.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

The finished medicinal product of Zalmoxis is defined as frozen donor T lymphocytes genetically modified with the SFCMM-3 Mut2 #48 retroviral vector and encoding for the HSV-TK and the ΔLNGFR genes in the final formulation medium and container closure system, ready for the intended medical use. The finished product contains 5-20x10^6 cells/mL, 10% DMSO as a freezing protectant and 7% human serum albumin (HSA) and ~87 mLNaCl solution (final volume depends on the number of cells), which are used as diluents (for freezing). The frozen cell product is filled in 50-500 mL ethylene-vinyl-acetate cryo bags, which are stored in stored liquid nitrogen. No cell washing is performed prior to patient administration.

The final DMSO concentration of 10 % in the finished product is considered to be acceptable.

The min-max volume and min-max number of cells of the finished product batches was not provided as it depends on the patient's weight. With a fixed final product concentration of 5-20 x 10^6 cells /mL finished product is divided into freezing bags (50-500 mL ethylen-vinyl-acetate cryo bags) with an appropriate volume depending on the total number of cells that are needed to obtain one dose of 1±0.2 x 10^7 CD3+/kg, including a 20% overhead to account for viability loss (historically around 20%) after thawing.

Manufacture of the product and process controls

Zalmoxis finished product is produced at MolMed SpA, Via Olgettina 58, 20132 Milan, Italy. The site is also responsible for the analytical testing and batch release of Zalmoxis.

For manufacture, the suspension of allogeneic T cells genetically modified are re-suspended in a solution of saline + HSA 7% + 20% DMSO). The final concentration of DMSO in the suspension is 10%.

The formulated cell suspension is then filled into the final container and frozen under a controlled temperature decrease ramp and finally stored in liquid nitrogen vapours.

The finished product is packaged as individual treatment doses in 50-500 mL ethylene-vinyl-acetate cryo bags and shipped to the clinical centres in liquid nitrogen. Temperature is monitored with a data logger to record excursions during shipment.

The potential process-related impurities, endotoxin, cell viability and % of non-T cell lineages are routinely monitored for finished product manufacture.

As the finished product is patient-specific therefore no reference standard material has been established. This is considered acceptable.

Product specification

The finished product manufacturing process is a continuous process from thawing the donor PBMCs to the production of the finished product, with no holding step at the level of the active substance. This is reflected in the limited set of active substance specifications. Therefore more emphasize is placed on the finished product release specifications, which include parameters for functionality, alloreactivity and immuno-phenotyping. The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with ICH guidelines.

During the procedure, the Applicant has revised the set of specifications and included GCV sensitivity and potency test to the specification set. The validation of methods will be completed post-marketing prior to commercial batch release. This proposal has been accepted.

Stability of the product

The proposed shelf life of the finished product is 18 months when stored in liquid nitrogen vapour.

Once thawed, the product should be administered immediately and in-use storage times and conditions should not exceed 2 hours at room temperature (15 - 30°C).

Based on the available stability data, shelf life and storage conditions as stated in the SPC are acceptable.

Comparability exercise for finished medicinal drug product

Adventitious agents

No viral clearance studies have been performed since the finished product is a cell therapy product which cannot undergo any elimination/inactivation virus step during the manufacture process.

An estimation of the risk of adventitious viral contamination in the finished product was provided. This is considered adequate.

An estimation of the presence of Replication Competent Retroviruses (RCR) and Endogenous Retroviruses in the finished product was provided. According to the Applicant, the risk of transmitting retroviruses to patients through the administration of contaminated doses of MM-TK is very low or negligible. The provided risk assessment was considered to be acceptable.

Adequate information on TSE has been presented for most of the materials. Information on viral safety of materials of animal origin used for MM-TK active substance, finished product or other relevant starting materials (e.g. for preparation of existing cell banks) are reported. Information on reagents

derived from human tissues used for MM-TK active substance or finished product manufacturing and formulation are provided as well.

GMO

Zalmoxis is defined as a GMO. For information on GMO and the detailed assessment of the Environmental Risk Assessment (ERA) please refer to the nonclinical section.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Zalmoxis is an advanced therapy medicinal product based on allogeneic genetically modified cells. It contains allogeneic T cells which have been genetically modified to express the Herpes Simplex Thymidine Kinase (HSV-TK) suicide gene and a truncated form of the human Low Affinity Nerve Growth Factor Receptor (ΔLNGFR) genes, which is intended to aid the manufacturing process by identifying transduced cells. Allogeneic T cells are obtained from lymphocyte aphaeresis of haploidentical donors.

During the procedure several major concerns were raised regarding the definition and control of the manufacturing process, comparability between the various stages of the manufacturing process and control of the finished product as well as a large number of other concerns.

During the procedure the Applicant provided a revised Module 3 including a process validation exercise for each process step with CPPs and CQAs involved. The CPPs for Zalmoxis active substance and finished product were identified as the parameters to impact CQAs. For each of the steps (including transduction and selection) the CPPs have been indicated and the process overall was considered to be sufficiently controlled. There were also concerns with respect to the optimisation of CPP ranges. However, as the Applicant proposed to set the ranges rather narrowly the process was considered well controlled.

It was also questioned whether comparability between the various stages of the manufacturing process had sufficiently been demonstrated. Several changes were introduced to the manufacturing process resulting in different processes A and B, which were used during clinical development and process C (including changes to the viral vector and media changes), which is the proposed commercial manufacturing process.

Specifically the Applicant was asked to provide characterisation data to demonstrate a direct comparison that the batches of the commercial process are comparable to the batches used in the clinical studies The applicant has provided data The Applicant has introduced finished product manufactured using vector produced according to a new vector manufacturing process C in the ongoing clinical study TK008. As this may further complicate interpretation of the clinical data introduction of this new vector manufacturing process had to be considered with great care because the history of manufacturing is already complicated. The Applicant provided additional data on validation (active substance and finished product), stability (vector, active substance and finished product), and comparability (vector, active substance and finished product). Adequate validation data were provided for vector manufacturing process C as well as for MM-TK manufacturing using vector from this process C. In addition stability was established.

A major concern was also related to the control of the finished product in particular the potency. To address these concerns, the applicant has performed additional tests to demonstrate the functionality of the T-cells. All quality issues are considered resolved. However, the Applicant has committed to address a number of recommendations on further quality development post-marketing. These are stated in section 2.2.6.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

In conclusion, based on the review of the quality data provided, the CAT considers that the marketing authorisation application for Zalmoxis can be approved from the quality point of view as all quality concerns have been resolved and the Applicant has committed to the list of recommendations as further detailed under 2.2.6.

The CHMP endorse the CAT assessment regarding the conclusions on the chemical, pharmaceutical and biological aspects as described above.

2.2.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CAT recommended several points for investigation.

The CHMP endorse the CAT assessment regarding the recommendation for future quality development as described above.

2.3. Non-clinical aspects

2.3.1. Introduction

The non-clinical program was discussed in a scientific advice EMEA/H/SA/516/2/2011/PA/SME/ADT/I.

Advanced Therapies Medicinal Product (ATMPs), due to their specificity, may follow a non-standard approach for non-clinical development. Indeed, paradigms described in the ICH M3 guideline that apply to non-clinical development of conventional pharmaceuticals may not be appropriate or relevant for Cell or Gene Transfer Medicinal Products (CGTMPs) or cell based therapy products consisting of genetically modified cells. In this light, it is also acknowledged that issues either related to proof of principle or toxicity data can be addressed concurrently in the same pre-clinical study(-ies) (EC/2009/120, 2009; ICHM3, 2009; ICHS6, 2011).

Preclinical in vitro and in vivo studies in immune deficient mice were performed using for cell preparation both the SFCMM-3 #35 retroviral vector and an optimized version of the vector, encoding a mutated form of the HSV-TK gene (HSV-TK Mut2 #48), as well as and an optimized process manufacture developed before launching the phase III clinical study. Moreover, NOD/SCID mice subcutaneously transplanted with human skin were also used so that the direct evaluation of allogeneic GvHD response against the grafted human skin could be observed.

2.3.2. Pharmacology

Primary pharmacodynamic studies

In vitro studies

In vitro pharmacodynamic and pharmacokinetic properties of the MM-TK were investigated in a series of studies performed at different stages of product development and encompassing either the original version of the SFCMM-3 retroviral vector encoding for the wild type HSV-TK gene (SFCMM-3 #35) or the optimized version coding for a mutated form of the HSV-TK gene (SFCMM-3 Mut2 #48).

Phenotypic characterization of MM-TK DS is integral part of routine Quality Control assessment performed on all produced batches. Batch analysis data indicate that > 99% of the cells express CD3,

expression of CD4 or CD8 is reversely related and variable depending on the batch. In addition, CD2, CD45 and CD95 are present on >99% of the cells while activation markers such as CD69, CD69L CD25 and HLA-DR are expressed at variable level between batches.

The relative distribution of memory T cells subsets was determined. Overall, the results obtained indicate that the transduced MM-TK lymphocytes were mostly T effector memory cells (T_{EM}). Unmanipulated PBL showed the highest percentage of naïve T cells (T_N) cells while T_{EM} and T central memory (T_{CM}) were similarly represented between MM-TK cells and unmanipulated T cells.

In addition, cell polarization following transduction into Th1 or Th2 type was evaluated based on cytokine production (IL-2, IFNγ, IL-4). An elevated percentage of transduced cells produced IFNγ and IL-2, cytokines typically produced by the Th1 and Tc1 lymphocytes. A lower percentage of cells produce IL 4, which is typically produced by the Th2 and Tc2 lymphocytes.

Response to ganciclovir

The HSV-TK protein makes donor lymphocytes sensitive to GCV in a reproducible, dose-dependent manner with an optimal percentage of cell death (Verzeletti et al., 1998). In vitro dependency of the MM-TK cell survival at different GCV concentrations is routinely assessed as part of the quality control assessment of manufactured batches. The dose/response relationships typically obtained is a five parameter logistic curve characterized by a two plateau trends at high and low GCV concentration and a steep linear trend in the central part of the curve in the range of 0.1 and 1.0 µM of GCV concentration. The specification limit for IC50 is ≤ 1.0 µM.

In vivo studies

Two studies in two different GvHD animal models both based on the NOD/SCID system were performed to evaluate the PK and primary PD of MM-TK. In preliminary studies a dose dependent GvHD-induced mortality was observed with a 100% incidence at the dose of $20x10^6$ cells/mouse, which was chosen as the target dose. Of note, the chosen target dose is around two log higher than the proposed clinical dose, thus providing large safety margins.

In these studies all animals were followed and observed for clinical signs effects on body weights, engraftment (human chimerism, evaluated as the percentage of human CD3+). At sacrifice human T cell infiltration in various organs was analysed by histopathology.

An arbitrary, global pathological score was designed to have a semi-quantitative overall evaluation of the severity of GvHD.

The first study utilised the humanized NOD/SCID mouse to evaluate MM-TK cell engraftment, long-term safety of the treatment and the efficacy of the suicide system in a xenograft model of GVHD. GvHD was recorded when >10% body weight loss and >1% donor chimerism. After GVHD diagnosis, mice were alternatively treated with GCV, PBS (as negative control) or euthanized. Body weight increase after the start of GCV treatment was eventually considered as a sign of efficacy of the salvage treatment.

Observations:

Body weight showed a general negative variation in all experimental groups in the first two weeks after treatment. The initial overall decrease of body weight observed in all groups was considered a direct consequence of the pretreatment protocol (irradiation). Only 10 days after treatment, body-weight loss, if associated with previous T cell engraftment was considered a relevant parameter for GvHD diagnosis. The body weight loss was more pronounced in the positive control group.

Chimerism increased progressively over time in all experimental groups.

GvHD occurrence was observed in all experimental groups, although at different rates. Positive control showed the higher incidence, while the MM-TK group had the lower incidence, while group 3 (other stimulation protocol) showed intermediate values.

All mice positively recorded for GvHD were either treated with GCV or with PBS (negative control) to evaluate the efficacy of the suicide machinery. At the end of the observation period, tissue sections were analysed for GvHD based on degree of transduced T cell infiltration. According to the study, data indicative of long term safety was obtained by evaluating the body weights of animals that never developed GvHD or that recovered after GCV treatment.

In the second study NOD/SCID mice were subcutaneously transplanted with 50 mm^2 of full-thickness human skin to allow a direct evaluation of alloreactive responses against the grafted human skin.

Observations:

Body weight. A transient and mild loss of body weight was observed only in the positive control. This is due to the low dose of γ irradiation used.

Chimerism increased progressively over time in the two treated groups. Maximum chimerism is observed 3 weeks after injection in both the unmanipulated cohort of mice, and the MM-TK treated mice.

Xenogenic GvHD incidence was monitored in all treated mice at week 1, 2, and 3 post-infusion and expressed as a score, which takes into account different observational parameters (body weight loss, ruffled fur, loss of animal activity and hunchback). Overall, the results showed the development of GvHD in both groups. An earlier onset, higher frequency and higher severity of GvHD was observed in positive control group when compared to MM-TK treated group.

The lower level of GvHD observed upon administration of MM-TK genetically modified cells in comparison to unmanipulated PBL is probably related to the lower content of naïve T present in the gene-modified lymphocytes.

Upon termination histological and immunohistochemistry evaluation of organs (liver, lungs, gut, kidney) and human skin was performed. The presence of human CD3+ cells was detected in almost all lymphohematopoietic organs, in other organs known to be targeted by the GvHD as well as in the allogeneic human skin.

Secondary pharmacodynamic studies

Studies on secondary pharmacodynamics have not been submitted (See discussion on non-clinical pharmacology).

Safety pharmacology programme

Studies on safety pharmacology have not been submitted (See discussion on non-clinical pharmacology).

Pharmacodynamic drug interactions

Specific studies on secondary pharmacodynamics safety pharmacology and pharmacodynamics drug interaction have not been performed.

2.3.3. Pharmacokinetics

To study the distribution of MM-TK cells, selected tissues were collected and processed for histology. Formalin-fixed, paraffin embedded organs were cut in 4 µm thin sections and stained with

haematoxylin and eosin for histological evaluation. Immunohistochemical assessment for the presence of human T lymphocytes was carried out by staining with anti-human CD3 mAb.

Engraftment was observed in the control mice as early as week 1 post-administration. An increase of the presence of human CD3+ cells in the course of the three weeks of observation was recorded not only in the positive control animals, but also in mice administered with the MM-TK test sample with levels raising from less than 10% in week 1 to around 30% or more at week 3. (see also Pharmacodynamics).

No formal biodistribution studies have been performed for MM-TK. (see discussion on non-clinical aspects).

No specific studies on viral shedding have been performed, however, since no direct in vivo administration of the SFCMM-3 Mut2 #48 retroviral vector is foreseen in the proposed therapeutic approach, shedding is limited to viral particles eventually associated to transduced T cells at time of patient administration. Due to the complexity and length of the manufacturing process, the final burden of free viral particles associated with the MM-TK would be probably very low. Furthermore, vertical germline transmission is unlikely to occur (see Environmental Risk Assessment).

2.3.4. Toxicology

To evaluate safety and functional properties of MM-TK, the Applicant developed an integrated approach which included both in vivo pharmacodynamic, toxicology and kinetic data concurrently obtained taking advantage of two different immunodeficient mouse models for Graft versus Host Disease (GvHD) based on the Non-Obese Diabetes/Severe Compromised Immunodeficient (NOD/SCID) mouse system, as well as series of in vitro laboratory tests mainly aimed at an in-depth investigation of the product.

Single dose toxicity

Single dose toxicity has been assessed in the immunodeficient animal models in the pharmacodynamic studies.

Repeat dose toxicity

Repeat-dose toxicity studies were not submitted (See discussion on non-clinical aspects).

Genotoxicity

Genotoxicity studies have not been submitted. (See discussion on non-clinical aspects).

Carcinogenicity

Studies aimed at the in vitro assessment of the oncogenic risk of MM-TK were performed by analysis of the TCR repertoire and of the integration profile of the retroviral vector. Further, oncogenic risk of MM-TK has been comprehensively evaluated taking into consideration also the results obtained from quality control of MM-TK batches so far produced since leukomogenesis may occur by other than insertional mutagenesis derived mechanisms.

In vitro assessment of oncogenic risk of MM-TK was performed by a series of ad hoc studies aimed to evaluate the clonality of the transduced cell population; characterize the insertional pattern of the SFCMM-3 #35 as well the SFCMM-3 Mut2 #48 retroviral vectors and any related variation in the transcriptional machinery; evaluate transduced cells survival in the absence of non-dispensable growth factor; assess any break out of replication competent retroviruses (RCR) which have been linked to leukomogenesis in immunosuppressed non-human primate (Donahue et al., 1992; Nienhuis et al., 2006).

Results obtained showed a cell population with no monoclonal or oligoclonal features. Additionally, supportive data confirming the clonal distribution of the transduced T cells were obtained by the spectratyping analysis of 25 Vβ families in follow-up samples of patients treated with MM-TK during the phase I/II. Median distribution of monoclonal, oligoclonal and polyclonal Vβ families was measured at immune reconstitution (n=17), 6 (n=9) and 12 (n=5) months after haematopoietic stem cell transplantation. The results obtained showed a T cell repertoire progressively developing towards a full polyclonality indistinguishable from that of healthy individuals at 1 year post transplant (Ciceri et al., 2009).

Insertional mutagenesis is a recognized safety concern of integrating vector-based Cell and Gene Therapy Medicinal Products (CGTMP) as serious adverse events linked to the haematopoietic system and due to insertional mutagenesis have been reported in 12 patients treated with gene corrected HCS transduced with γ-retroviral vectors (Aiuti et al., 2012; Gaspar et al., 2011a; Hacein-Bey-Abina et al., 2008; Hacein-Bey-Abina et al., 2010; Hacein-Bey-Abina et al., 2003; Seymour and Thrasher, 2012; Stein et al., 2010). It has been showed that retroviral vectors do not integrate in the human genome randomly. On the contrary, their integration pattern is skewed towards specific genomic regions, called Common Insertional Sites (CIS), which include actively transcribed genes. Moreover, all transformed cell clones harboured vector insertions next to proto-oncogenes like LMO-2 or Evi1 leading to their activation that subsequently progressed to malignancy (Biasco et al., 2012; Hacein-Bey-Abina et al., 2003; Ott et al., 2006; Stein et al., 2010).

The detection of integration nearby cancer genes does not necessarily associate to malignant clonal expansion as in ADA-SCID trial patients harbouring integrations targeting the LMO2 or MECOM did not aberrantly expand (Aiuti et al., 2012; Candotti et al., 2012; Gaspar et al., 2011b) thus pointing out that insertional mutagenesis and transcription deregulation of cellular genes are not sufficient per se to induce malignant transformation and that other factors are important and synergistically cooperate to increase the risk of oncogenesis, including cell differentiation stage. In fact, oncogenesis appears inversely related to the maturity of the cell as no adverse events related to vector integration have been observed both in vitro (Newrzela et al., 2008) and in all the clinical trials based on ex vivo gene transfer into mature T cells (Deeks et al., 2002; Mitsuyasu et al., 2000; Recchia et al., 1999; Walker et al., 2000), thus suggesting that mature T cells are less susceptible to transformation than HSCs likely because of the different genetic program of the two cell types (Biasco et al., 2011).

However, even if T cells can be considered a safer substrate for genetic modification by γ retroviral vectors, integration analyses and expression profile was performed in three SFCMM-3 Mut2 #48 transduced cell batches. Moreover, the same analysis was performed on four SFCMM-3 #35 clinical batches and on the follow-up samples taken 1 month up to 9 years after patient administration, to assess whether any selection may occur in vivo post-treatment. Analyses were performed by LM-PCR followed by sequence mapping using BLAST browser. Additionally, in order to verify whether the level of gene expression influences the ability of the retroviral vectors to integrate in specific genome sites, the expression profile of >16.000 genes in mock-transduced lymphocytes harvested at the time of transduction, was analysed by microarray analysis. Overall, the results obtained showed that, despite the preferential integration of the retroviral vector within or in the proximity of transcriptional active genes, the transduced T cell population maintained stable gene expression profile, phenotype and biological functions. A comparison of the integration sites in transduced T cells before and after infusion showed that vector integration within genes involved in cell cycle control or in other physiological T cell functions are counter-selected in vivo. Furthermore, no clonal selection or expansion could be observed during the follow-up. Therefore, the results obtained clearly point out that retroviral integration in SFCMM-3 transduced T cells is not associated with a measurable risk of insertional oncogenesis (Recchia et al., 2006). The vector copy numbers as reported for a few clinical batches is relatively low.

During the course of the MM-TK development, at least 115 independent preparations of human lymphocytes transduced with 2 different retroviral vectors (84 with SFCMM-3, 25 with SFCMM-3 Mut2 and at least 20 with an unrelated vector that does not contain HSV-TK) have been tested for IL-2 dependency. This assay calls for the cultivation of test cells in the presence or absence of IL-2 for 30 days. Periodically, the cell number and viability using trypan blue is determined. In all cases analysed the genetically modified lymphocytes were IL-2 dependent.

In vivo data

The MM-TK DP is a species specific product and therefore standard carcinogenicity studies using rodents or non-human primates as described in the ICH guidelines are not appropriate. Therefore, no specific in vivo carcinogenicity studies were performed for the MM-TK to date.

However, based on a supposed, highly context dependant oncogenic activity of the ΔLNGFR (Baum et al., 2003), both pre-clinical and clinical evidence supporting the safety of ΔLNGFR as a cell-marking molecule has been accumulated in a collaborative effort between 17 independent groups of investigators, (Bonini et al., 2003). Cumulative data obtained from >300 mice transplanted with BM cells transduced with ΔLNGFR-expressing retroviral vectors showed normal engraftment, persistence and differentiation of ΔLNGFR-expressing haematopoietic stem cells (HSC) in primary, secondary and tertiary haematopoietic cell transplant (HCT) recipients, with no adverse events. Over 100 of these mice were monitored for >20 weeks after HCT; more than 70 animals, including 16 recipients of secondary or tertiary HCT, were monitored for > 28 weeks. Considering that a total of $>1x10^9$ transduced cells were transplanted, and assuming an average of one retroviral integration per cell, the estimated risk of oncogenic transformation after transduction with a HSV-TK/ΔLNGFR-encoding retroviral vector is <1 in 10^9 integration events.

Moreover, transcriptosome studies recently performed to investigate the influence of cell activation and selection process, further support the absence of oncogenic potential of transduced T cells, as the major impact of ex vivo T cell activation on oncogene expression was down-regulation with no case of up-regulated oncogene expression (Deschamps et al., 2008). In detail, the analysis showed that no impact on the Trk/NGFR expression pathway was observed after selection as previously suggested (Baum et al., 2003), but also expression of LMO-2 and MDS-Evi1 genes, so far found involved in insertional mutagenesis phenomena (Hacein-Bey-Abina et al., 2003; Ott et al., 2006; Stein et al., 2010) in patients administered with genetically modified CD34+ stem cells, was unchanged.

Toxicokinetic data

Local Tolerance

Local tolerance studies were not submitted. (See discussion on non-clinical pharmacology).

Other toxicity studies

Studies on antigenicity or immunotoxicity, dependence, metabolites, impurities were not submitted (See discussion on non-clinical pharmacology).

2.3.5. Ecotoxicity / environmental risk assessment

The environmental risk assessment was performed in accordance with Annex II to Directive 2001/18/EC on the deliberate release into environment of genetically modified organisms (GMOs) and following the precautionary principle using the methodology set down in Commission Decision 2002/623/EC, and EMA guidelines on environmental risk assessments for medicinal products consisting of, or containing GMOs (EMEA/CHMP/BWP/473191/2006).

A complete ERA included as part of the submission of the MAA discusses the environmental risk assessment for the clinical use of Zalmoxis.

Zalmoxis is constitued of genetically modified lymphocytes *ex vivo* transduced with a γ retroviral vector. The viral vector used for *ex vivo* transduction is replication defective and therefore unable to replicate when integrated in the final host cells. No *in vivo* administration of the retroviral vector is foreseen, but *ex vivo* genetically modified T cells are administered.

The assessment is performed in taking into account the indications described in the Directive 2001/18/EC on the deliberate release of GMO's and EMA guidelines on environmental risk assessments for medicinal products consisting of, or containing genetically modified organisms (GMOs) (EMA/CHMP/BWP/473191/2006) and on scientific requirements for the environmental risk assessment of gene therapy medicinal products (EMA/CHMP/GTWP/125491/2006).

The Applicant developed two SFCMM-3 retroviral vectors: the SFCMM-3 #35 retroviral vector encoding for the WT form of the HSV-TK gene and its final, subsequent version, the SFCMM-3 Mut2 #48 retroviral vector encoding for a mutated form of the HSV-TK gene, the latter is considered the GMO central to this ERA. Similarly, the two vectors were used to prepare clinical batches of MM-TK cells, either used in the Phase I/II TK007 clinical trial or in the subsequent Phase II TK008 study.

The SFCMM-3 Mut2 #48 retroviral vector is an integrating retroviral vector, replication defective by design. Moreover, the preparation of the medicinal product is performed by ex vivo transduction and therefore no in vivo administration of the viral vector is foreseen.

The target cells for transduction are donor T cells. These are terminally differentiated cells with migratory properties likely directed towards lymphohaematopoietic organs as also indicated in gene marking studies performed in the frame of non-clinical development. Vertical transmission may only occur with infection and stable integration of the viral vector (or viral vector related organisms) in germinal cells. The chance that this event happens in the proposed setting is essentially related to viral shedding or to the probability that a new virus able to carry out a complete life cycle arise and then migrates towards sexual organs.

No specific studies on viral shedding have been performed. However, since no direct in vivo administration of the SFCMM-3 Mut2 #48 retroviral vector is foreseen in the proposed therapeutic approach, shedding is limited to viral particles eventually associated to transduced T cells at time of patient administration. Due to the complexity and length of the manufacturing process, the final burden of free viral particles associated with the MM-TK would be probably very low.

Since the proposed therapy is indicated as adjunctive treatment to haematopoietic cells transplantation in leukaemia affected patients, individuals administered with MM-TK have been previously treated with highly aggressive myeloablative treatments that are associated with sterility, thus excluding any possible vertical transmission of vector related sequence to the progeny.

In summary vertical germline transmission is unlikely to occur since:

- The provirus is integrated in the final human cell host and mobilization of the virus is needed to produce an RCR able to infect patient tissues and ultimately germline cells. The likelihood that this happens is minimized by the fact that at least three different recombination events are required to produce an RCR;
- Transduced T cell fate following infusion is mainly driven by stimulation/culture conditions used for manufacturing but ultimately targeted to hematopoietic and not sexual organs
- The limited number, if any, of free infectious viral particles associated with the MM-TK cells will be rapidly inactivated by the complement system of the patient;

- The myeloablative conditioning regimens performed in the context of a haploidentical transplant of selected CD34+ cells (and included in TK008 study) is associated with sterility.

Based on the above, the MM-TK product falls in the lower risk category and therefore, in the light of recommendations reported in the EMA document related to inadvertent germ line transmission of gene therapy vectors (EMA/273974/2005, 2007), no specific studies have been performed to evaluate germ line transmission of SFCMM-3 Mut2 #48 related sequences.

Information on the characteristics of Zalmoxis and its components, on the intended mode of administration of Zalmoxis and on possible interaction between Zalmoxis and the environment has been used to evaluate the environmental risk of Zalmoxis, in particular any potential adverse effects due to survival, multiplication or dispersal, in case it would get into contact with people other than patients and the environment.

From the environmental risk assessment it was concluded that the risk to people other than patients and the environment from the intended marketing of Zalmoxis is negligible. Potential adverse effects and negative consequences, such as the potential of lymphoproliferation due to a release of significant numbers of free retroviral vectors or RCR and infection of non-target human and animal species were found unlikely to occur because of the specificity of the medicine and of the poor likelihood of RCR break out.

2.3.6. Discussion on non-clinical aspects

In vitro analysis of the transduced T cell population indicates that there is a shift in the T cell population as a result of the transduction protocol in which T cells were also stimulated with anti-CD3 when compared to untreated/stimulated PBMCs. This shift included inversion in the CD4/CD8 ratio among the CD3+ T cell population, enrichment of effector memory cells, primarily at the cost of frequency of naive T cells, elevated percentage of cells producing Tc1 cytokines. It was shown that TK-cells infusion in patients promotes a rapid immune reconstitution and a direct control of viral infection and leukemic relapses. (Vago et al. Blood 2012). Furthermore, it was shown preservation of alloreactive and of viral-specific precursors along the transduction process in batches manufactured with both the proposed commercial manufacturing process or with other process (Marktel et al. Blood 2003).

MM-TK cells are genetically modified to express the MM-TK suicide gene, which, in theory will allow treatment of the patients in case of adverse events caused by MM-TK cells, most likely graft versus host disease (GvHD). The TK gene is activated by ganciclovir (GCV). Based on the batch analysis it appears that a sufficient level of GCV sensitivity is reached for the product. However, it is not clear how these in vitro results relate to the in vivo response of circulating MM-TK cells to GCV. Newly submitted Clinical data show that in the majority of patients GCV treatment at time of GvHD results in a reduction in circulating LNGFR+ cells at the 4th day of GCV treatment. This indicates that GCV treatment works. The clinical data suggest that it is likely that GCV treatment contributed to the resolution of GvHD. Provided that vector #35 and #48 only differ one base pair that does not influence aminoacid sequence of the TK gene, data obtained with vector #35 could be finally used for #48 as well. In four publically available non clinical studies the effect of combination of GCV treatment and the insertion of the HSV-TK gene in the donor cells on GVHD is described. Insertion of the HSV-TK gene in donor cells combined with GCV treatment resulted either in delay of lethal GVHD, in increased survival or in the residual presence of human CD45RA+ CD3+ cells. Thus, preclinical, GCV treatment adds an effect to the introduction of HSV-TK in the donor cells.

Two studies in two different GvHD animal models both based on the NOD/SCID system were performed to evaluate the PK and primary PD of MM-TK. 1: humanized NOD/SCID mouse to evaluate

MM-TK cell engraftment and the efficacy of the suicide system in a xenograft model of GVHD, and 2: humanized NOD/SCID mice that were subcutaneously transplanted with 50 mm2 of full-thickness human skin to allow a direct evaluation of alloreactive responses against the grafted human skin. In these studies MM-TK was compared to unmanipulated PBMCs. In both studies the engraftment and potency to induce GvHD was less for MM-TK cells. Both the reduced GvHD and reduced T-cell engraftment are the effect of the required T-cell activation, which promotes a differentiation of T-cells to effector and effector memory cells. This supports the notion that the transduced cells are not directly comparable to unmanipulated PBMCs.

Only in the first model the efficacy of GCV treatment was evaluated. However, as only a limited number of animals developed GvHD/Xeno-reaction only limited information on the effectiveness of GVC treatment could be obtained in this study. However, the submitted clinical data showing that in the majority of patients GCV treatment at time of GvHD results in a reduction in circulating LNGFR+ cells at the 4th day of GCV treatment, indicates that GCV treatment and thus addition of the transgene results in reduction of MM-TK driven GVHD.

There are no specific studies on secondary pharmacodynamics and safety pharmacology, which is agreed considering the type of product.

It is recognized that standard pharmacokinetics studies are not applicable for this type of product. Distribution of MM-TK cells was evaluated in the two in vivo studies described in the pharmacology section. However interpretable results can only be obtained in the study where GvHD was not treated by GCV. MM-TK cells were tracked with a focus on lymphohaematopoietic and non-lymphohaematopoietic organs known to be target of GvHD. Overall, the results obtained showed that the presence of human CD3+ cells was detected, three weeks post-dosing, in almost all lymphohaematopoietic organs, in other organs known to be targeted by the GvHD, as well as in the allogeneic human skin. t is noted that the data on biodistribution is limited to relatively few organs and limited number of animals. Organ selection was based on the frequency with which the organs are affected by GvHD (The EBMT handbook 6th Edition, 2012, Chapter 13: GvHD by Jane Apperley and Tamas Masszi). Organs spared from GvHD (in particular CNS, pancreas, stomach, ovaries) were excluded from the analysis. The available data indicate that the cells can end up in any tissue which is not an unexpected finding following iv administration.

MM-TK cells express ΔLNGFR at their cell surface. This truncated protein represents a surface marker for transduced cells lacking a functional (cytoplasmic) domain. However, the extracellular domain of the protein is still present. It is not clear whether this extracellular domain can still interact with its (natural) ligands, which may affect the distribution of transduced cells especially when these ligands are membrane bound proteins. Given the fact that NGF, the potential ligand for NGFR, is a secreted protein and not membrane bound, is unlikely to affect localisation of the MM-TK cells.

Germline transmission of the integrated transgene is not an expected event because of the provirus is integrated in the final human cell host and mobilization of the virus is unlikely to occur and the limited number, if any, of free infectious viral particles associated with the MM-TK cells are expected to be rapidly inactivated by the complement system of the patient.

Lack of specific pharmacokinetic drug interactions is agreed considering the type of the product, and no additional pharmacokinetic studies are needed.

Studies presented in the pharmacology section for in vivo safety evaluations are severely hampered by lack of data (end points), insufficient and inconsistent reporting/documentation. The DLI engraftment is dependent on the availability of human cytokines IL7, IL15 and IL2, and promoted by the TCR of HLA-expressing antigen presenting cells, which are not present in the animal model. The extrapolation of the repeated dose administration in infused mice to human is limited.

In the toxicology section the concern for oncogenicity has been addressed. Considering the nature of the product, genetically modified T cells, it is agreed that the occurrence of GvHD and oncogenic transformation are the two main concerns associated with this product.

The potency of Zalmoxis to induce GvHD has been addressed in the studies also presented in the pharmacology section. Some safety related endpoints (death, bodyweight loss) were included in this study, however they have not been clearly described in the study report.

Oncogenicity is more than a theoretical concern as leukaemia-like lymphoproliferative disorders have been observed in patients receiving retrovirally transduced HSC. The Applicant has performed studies aimed at the in vitro assessment of the oncogenic risk of MM-TK. These studies were performed by analysis of the TCR repertoire and of the integration profile of the retroviral vector. The in vitro studies pertain 1) evaluation of clonality of the transduced cell population, 2) characterisation of the insertional pattern, 3) evaluation of growth-factor independent growth, and 4) assessment of break out of replication competent retroviruses (RCR). Overall the presented and discussed results do not suggest an oncogenic risk.

The lack of studies on reproductive and development toxicity is justified. No discussion on antigenicity was provided.

The environmental risk of Zalmoxis is negligible.

As expected, in vitro and in vivo data indicate that the MM-TK are not the same as unmanipulated PBMCs. A skewing of the T cell population towards CD8+ T effector memory cells of Th1 type is observed. Moreover, the engraftment and potency to induce GvHD in vivo was less for MM-TK cells when compared to unmanipulated PBMCs. The impact of these changes on the functionality of the T-cells has not been fully elucidated, but T-cell responsiveness against a-specific and antigen specific stimuli was shown to be maintained in the MM-TK product.

The CHMP endorse the CAT discussion on the non-clinical aspects as described above.

2.3.7. Conclusion on the non-clinical aspects

Conventional toxicology, carcinogenicity, mutagenicity and reproductive toxicology studies have not been performed, which is considered acceptable.

Non clinical safety data obtained in two different immunedeficient animal models for Graft Versus Host disease did not indicate special hazards for humans, but allowed only a very limited safety assessment. In vitro evaluation of oncological potential indicate that the risk of malignant transformation is low.

Based on the submitted data and in the context of an ATMP the non-clinical aspects of the product can be considered sufficiently described.

The CHMP endorse the CAT conclusions on the non-clinical aspects as described above.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the European Union were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Table 1: Overview of the studies supporting the MAA

Study ID	No. of study centres/	Design	Study Posology	Study Objective	Subjs by arm entered/ compl.	Duration	Gender M/F Median Age	Diagnosis Incl. criteria	Primary Endpoint
TK007 (compl.)	8 (IT, UK, DE, GR, IL)	Open-label Single arm Phase I/II	MM-TK cells $1x10^6$/kg or $1x10^7$/kg IV Up to 4 times every 30 days until IR	IR Control of GvHD-GvL effect	57/52	11 y*	23/30 49 y (17 - 66)	High-risk haemato-logical malignancies candidate for haplo-identical HSCT	IR
TK008 (ongo.)	16 (IT, DE, FR, E, GR, B, IL, USA)	Open-label Rando-mised Phase III	MM-TK cells $1x10^7$/kg IV Up to 4 times every 30 days until IR	Superiority versus standard haplo-identical HSCT	29/17 Planned 170	7 y	11/6 49 y (19 - 65)	High-risk AML and ALL in first or subsequent remission or in relapse candidate for haplo-identical HSCT	DFS/ PFS

** enrolment 6 years, follow-up 5 years*

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; B, Belgium; compl, completed; DE, Germany; E, Spain; FR, France; GR, Greece; GvHD, Graft-versus-Host Disease; GvL, Graft versus Leukaemia; HSCT, haematopoietic stem cell transplant; IL, Israel; IR: immune reconstitution; IT, Italy; IV, intravenous; ongo, ongoing; UK, United Kingdom; USA, United States of America; y, years.

The application for marketing authorisation was initially based on the single arm, phase I/II study TK007 with the primary objective of immune reconstitution (IR) defined as CD3+ cells >100 per µL by MM-TK treatment. The randomised controlled phase III study TK008 with disease free survival (DFS)/progression free survival (PFS) as primary endpoint, is ongoing.

Upon assessment of the TK007 data and as only limited data from the TK008 study were available, the applicant was asked to perform a comparison of the MM-TK treated patients (TK007 and TK008 combined) with results from suitable historical controls to determine the adjunctive treatment effect of the MM-TK cells given after T-cell depleted haploidentical hematopoietic stem cell transplantation (HSCT). To this end, the Applicant formally approached the European Blood and Marrow Transplant

(EBMT) society (www.ebmt.org) to request the use of their patient database to compile an appropriate control group.

This control group included patients who underwent haploidentical transplantations performed according to the two most commonly used modalities of GvHD prevention: T-cell depleted transplant without any add-back strategy (TCD cohort) and T-cell replete (unmanipulated) transplant followed by post-graft infusion of cyclophosphamide and immune suppression with a calcineurin inhibitor and mycophenolate (PT-Cy cohort). Both options are also included in the control arm of the ongoing phase III trial TK008.

In total 47 patients have been treated with MM-TK cells (n=30 in TK007 and n=17 in TK008) of whom of 45 patients clinical data were available (n=30 in TK007 and n=15 in TK008). For these patients, matched control patients were identified in the EBMT database using a specific set of matching parameters.

The results of the matching strategy and the comparison between the MM-TK treated population and the compiled control population are presented and discussed in the further below section *Clinical efficacy*.

2.4.2. Pharmacokinetics

Zalmoxis (MM-TK) consists of T lymphocytes that are harvested from the same donors as the CD34+ cells used for HSCT, and are ex vivo genetically modified to express a mutated form of the HSV-TK suicide gene. The purpose of the HSV-TK suicide gene is that if MM-TK cells evoke a GvHD reaction, the administration of GCV results in the selective elimination of proliferating MM-TK cells and thereby in GvHD control. For identification and cell selection purposes, the T lymphocytes are also genetically modified to express the human truncated low affinity Nerve Growth Factor receptor (ΔLNGFR).

The relative distribution of memory T-cells subsets appeared different (see non-clinical assessment report) between the grafts produced using retroviral #35 (process A – TK007) and #48 (process B+C – TK008) as determined using a specific set of surface markers. The clinical significance of these differences in the T-cell composition of the products in relation to achieving immune reconstitution (IR) was investigated.

The number of samples available was limited (n=13 for TK007 and n=16 for TK008) and the variability in the composition of the T-cell subsets was large. In this respect, products that were made using process A seem to contain a median ± 2-fold lower percentage of naïve T-cells than the grafts produced using process B+C, though ranges are wide and overlap, i.e. 10.3% (range 7.2 – 39.6) vs 23.9% (0.9 – 63.7%), respectively. Regarding the ratio EC/TM, this appeared median ± 5-fold lower in the products for the patients in TK007 (process A) as compared to the products for the patients in TK008 (process B+C), i.e. 0.48 (0.11 – 1.91) vs 2.36 (0.39 – 5.59), but also here ranges are wide and almost completely overlap. The differences were not statistically significant. There was no correlation between the T-cell composition in the grafts and the median number of cells administered to patients from TK007 and TK008. There were no clear differences in the reported baseline characteristics between the groups and the results further showed that when using the T-cell subset data (naïve vs central memory (CM) vs effector memory (EM) as continuous or as dichotomous variables, there was no correlation with IR (as illustrated for the dichotomous results in table 19).

Table 2: Median values of T-cell subsets according to IR

	Median values of T-cell subsets according to IR			
	TK007 (n=13)		TK008 (n=16)	
	IR	No IR	IR	No IR
T-Naive	11.4%	10.5%	18.8%	29.3%
T-CM	19.0%	23.4%	41.3%	49.2%
T-EM	67.6%	66.0%	33.1%	30.5%
CM/EM ratio	0.49	0.51	2.46	2.32
p-value=nonsignificant for all tests				

As the product is constituted of cells, i.e. donor T lymphocytes, no PK-PD studies have been conducted. Circulation of normal and genetically modified T lymphocytes was measured and Spearman correlation between Cmax LNGFR+ and Cmax HSV-TK transgene PCR was 0.76. However, HSV-TK gene and LNGFR expression are regulated by different promoters and also, the different analysis methods, i.e. flow cytometry (LNGFR) vs PCR (HSV-TK), may hamper a perfect correlation.

Starting from first month after the baseline, a progressive increase of T-cells was observed. The percentage of LNGFR+ positive CD3+ cells decreased over time after MM-TK infusion, because the number of CD3+ cells increased, while the number of LNGFR+ CD3+ cells was rather stable during the 8 month follow-up period. The progressive increase in LNGFR negative CD3+ cells and the change in relative distribution pattern of immune phenotypes over time, showing an increase in effector cells instead of effector memory cells and an increase in naïve cells, suggest a thymus-dependent T-cell development.

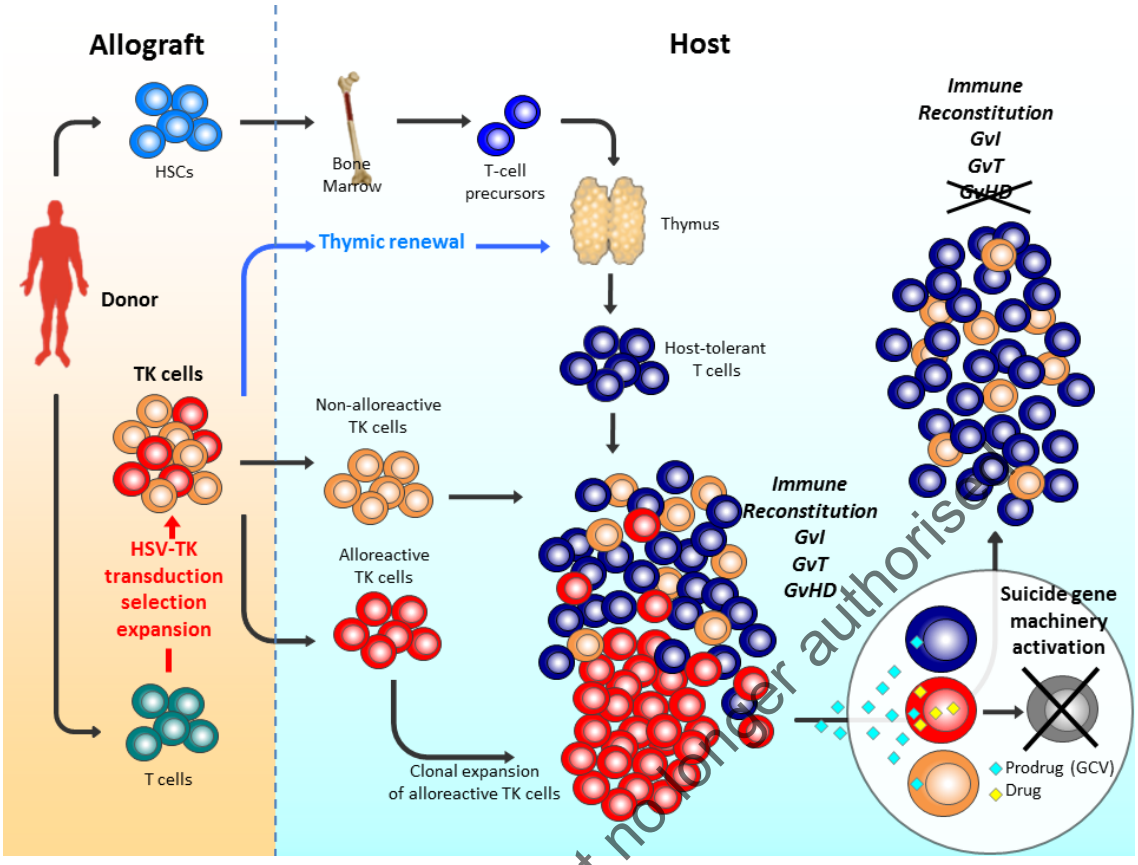
2.4.3. Pharmacodynamics

Mechanism of action

The proposed mechanism of action of MM-TK cells is depicted in Figure 4. After a myeloablative conditioning, patients receive CD34-selected donor's hematopoietic stem cells. T lymphocytes, harvested from the same donors, are *ex vivo* genetically modified to express a mutated form of the Herpes Simplex Virus Thymidine Kinase (HSV-TK) suicide gene. For identification and cell selection purposes, the T lymphocytes are also genetically modified to express the human truncated low affinity Nerve Growth Factor receptor (ΔLNGFR).

Gene modified T cells are purified and infused after haplo-HSCT. The proposed in-vivo fate of gene modified cells is represented: non-alloreactive (orange circles) and alloreactive (red circles) T cells directly mediate a rapid immune reconstitution and prompt a thymic-dependent long-term immune reconstitution, composed of host tolerant (black circles) cells. If TK- cells evoke a GvHD reaction, the administration of ganciclovir may result in the selective elimination of proliferating TK-cells and in GvHD control. Thus, the in-vivo activation of the suicide gene machinery allows abrogation GvHD, while preserving the recovery of a functional and wide T-cell compartment.

Figure 1: Mechanism of action of MM-TK cells



Primary and Secondary pharmacology

Gene-marking and immunophenotype characterization in patients following MM-TK infusion (study TK-007)

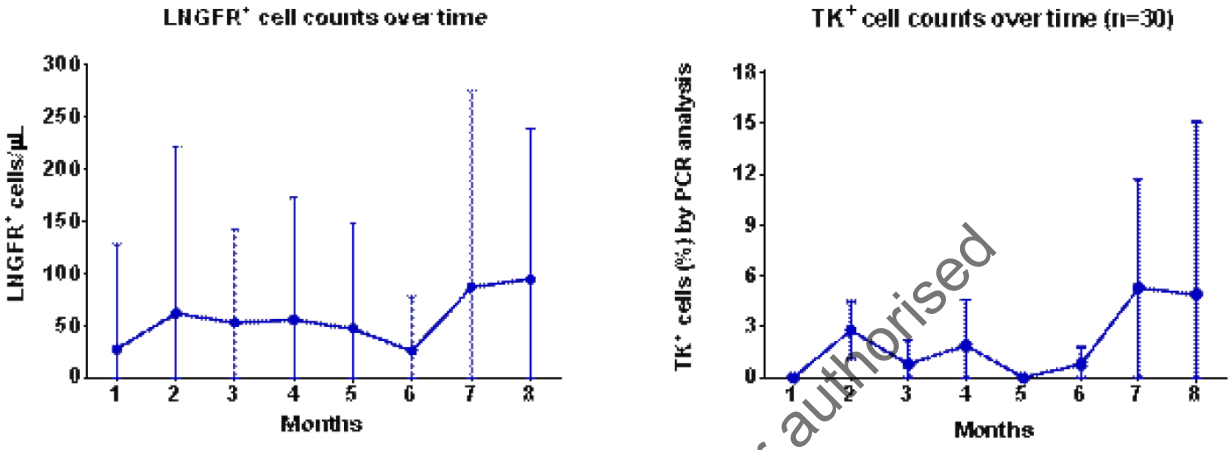
The infused cell products were characterized in terms of cell subsets by the immune phenotype profile (see table 20). MM-TK cells expressed the panlymphocytic CD2 marker and the T-cell CD3 marker, whereas B lymphocytes (CD19+) and monocytes (CD14+) were lost during culture. Immune phenotype analyses showed that all transduced MM-TK cells were CD45+ and CD3+ T-cell subpopulations. CD4+ and CD8+ were represented in variable degrees among different donors, with a general prevalence of CD8+ cells. Cells with CD56 marker, typical of natural killer (NK) cells and some subpopulation of T cells, were also present. Overall, about 95% of the cells were LNGFR+ cells, i.e. transduced MM-TK cells.

Table 3: Immunophenotype characterization of the infused cell products

Value	$CD45^+$	$CD3^+$	$CD4^+$	$CD8^+$	$CD8/CD4^+$ ratio	$CD56^+$	$CD2^+$	$CD16^+$	$LNGFR^+$
Mean	99.5%	96.9%	32.7%	70.1%	2.6	13.1%	98.7%	2.0%	94.5%
Median	99.7%	97.5%	31.6%	71.1%	2.2	11.1%	98.9%	1.9%	95.0%
Minimum	96.8%	88.8%	15.2%	44.4%	0.8	4.0%	95.8%	0.5%	90.0%
Maximum	100%	99.0%	95.6%	85.0%	5.4	32.7%	99.9%	4.7%	98.3%
Data from 29 patients receiving 47 infusions; values for two infusions of patient TK21 are missing									

Engraftment of MM-TK cells was quantified by flow cytometry analysis evaluating the LNGFR expression on circulating lymphocytes and by PCR analysis evaluating the HSV-TK transgene on DNA extracted from circulating peripheral blood mononuclear cells (PBMC). Concentration-time profiles of LNGFR+ and TK+ cells are shown in Figure 5. Spearman correlation between Cmax LNGFR+ and Cmax HSV-TK transgene PCR was 0.76.

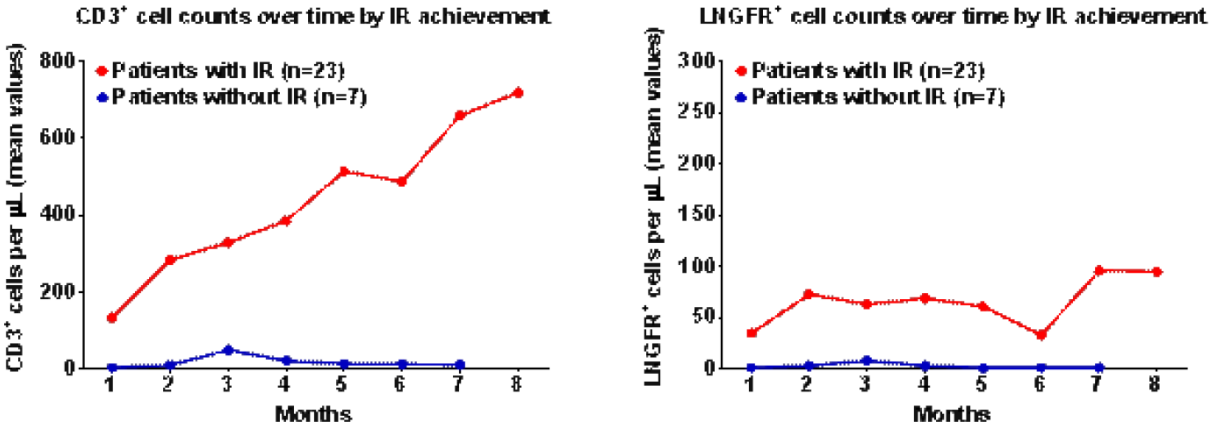
Figure 5: Mean (± SD) distribution values over time of LNGFR+ and TK+ cells in treated patients (study TK-007)



Starting from first month after the baseline a progressive increase of T cells was observed (Figure 5). Mean values increased above the cutoff value of 100 cells/µL for CD3+ lymphocytes. During the immunologic follow-up of treated patients, an increase in percentage of CD3+ cells that were negative for the surface marker LNGFR was detected. CD8+ increased over time doubled in relation to CD4+ cells, as expected based on the relative distribution of T-cell subsets in the infused cell products. The median time to reach a count ≥ 50 cells/µL for CD4+ cells was 88 days from the HSCT and 27 days from last infusion, while for the CD8+ cells the corresponding values were 77 and 21, respectively.

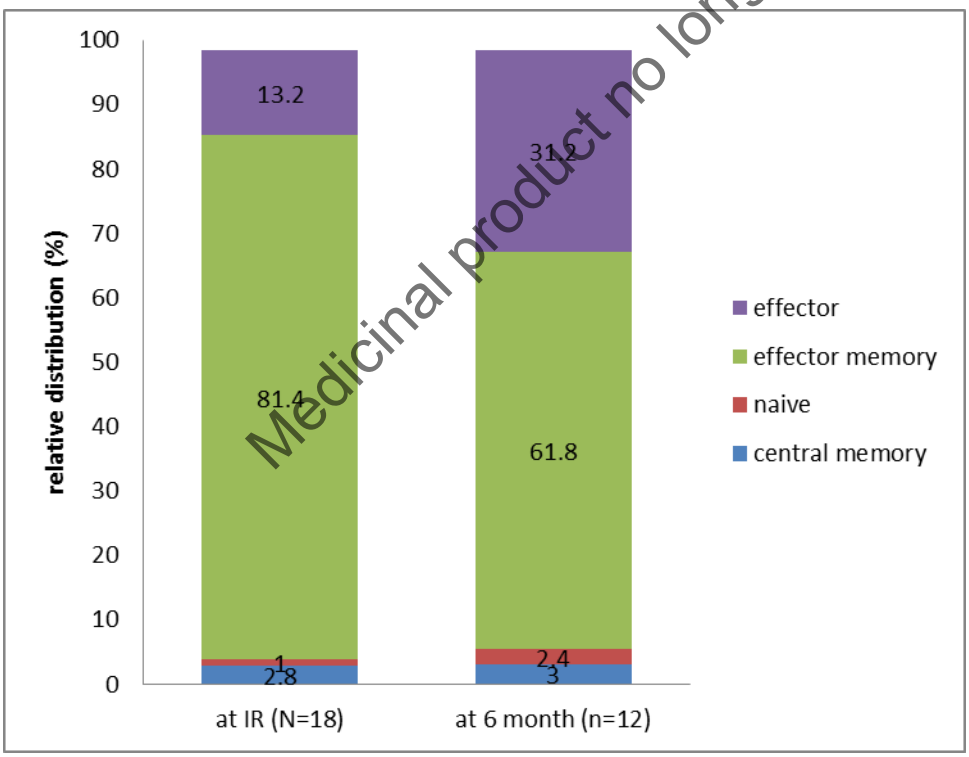
Circulating CD3+ and LNGFR+ cell counts were significantly higher for 23 patients who achieved IR than for 7 patients who did not. Median Cmax values for CD3+ were 591 cells/µL (95% CI 434-872 cells/µL) and 27 cells/µL (95% CI 0-82 cells/µL) in subjects with and without IR, respectively, and median Cmax for LNGFR+ cells were 105 cells/µL (28-253 cells/µL) and 9 cells/µL (0-15 cells/µL), respectively. The median time to reach peak values was 76 days (95% CI 55-150 days) for subjects with IR and 43 days (95% CI 0-81 days) for subjects without IR.

Figure 6: Concentration-time profiles of CD3+ and LNGFR+ cells according to immune reconstitution achievement



After a first wave of circulating MM-TK+ cells, the majority of T cells supporting long-term immune reconstitution did not carry the suicide gene. At IR nearly all circulating CD3+ cells had an effector-memory phenotype similar to that of infused cells but at 6 month the percentage of naïve and effector lymphocytes were increased (see Figure 7).

Figure 7: Mean distribution values of immune phenotypes of CD3+ cells in peripheral blood over time



2.4.4. Discussion on clinical pharmacology

The application is based on one single arm study TK007. The primary objective of study TK007 is IR that is defined as CD3+ cells >100 per µL upon MM-TK treatment. The product is constituted of donor T lymphocytes genetically modified ex vivo to express the suicide gene HSV-TK gene and the surface

marker LNGFR. As the product consists of genetically modified donor T lymphocytes, no classical PK-PD studies have been conducted. However, circulation of normal and genetically modified T lymphocytes was measured, which is considered acceptable. Relationships between dose and GvHD are discussed in the safety section of this report.

In study TK007, the MM-TK cells were produced using the original version of the SFCMM-3 retroviral vector encoding for the wild type HSV-TK gene (SFCMM-3 #35 – process A), while in study TK008 the optimized version coding for a mutated form of the HSV-TK gene (SFCMM-3 Mut2 #48) was used. This change seemed to affect the distribution of the T-cell subsets. However, analysis of T-cell subset of n=13 for TK007 and n=16 for TK008 from the in total 47 patients treated with MM-TK, showed that the variability in the composition of the T-cell subsets is large and that the observed difference in the median percentage of naïve T-cells and the EC/TM ratio was not statistically significant. Most importantly there was no correlation between the T-cell subset composition of the grafts and the achievement of IR. The impact of the missing samples is likely to be limited due to the large interpatient variability in T-cell profile.

The number of LNGFR+ cells was rather stable during the 8-month follow-up period. Spearman correlation between Cmax LNGFR+ and Cmax HSV-TK transgene PCR was 0.76. HSV-TK gene and LNGFR expression is regulated by different promoters. The different analysis methods, i.e. flow cytometry (LNGFR) vs PCR (HSV-TK), may hamper a perfect correlation, but presence of the HSV-TK gene is essential for this treatment.

Starting from the first month after the baseline, a progressive increase of T-cells was observed. The percentage of LNGFR+ positive CD3+ cells decreased over time after MM-TK infusion, because the number of CD3+ cells increased, while the number of LNGFR+ CD3+ cells was rather stable during the 8-month follow-up period. The increase in LNGFR negative CD3+ cells and the change in relative distribution pattern of immune phenotypes over time, showing an increase in effector cells instead of effector memory cells and an increase in naïve cells, suggest a thymus-dependent T-cell development.

The primary mechanism of action of Zalmoxis relies on its ability to engraft and stimulate immune-reconstitution.

Zalmoxis is constituted of donor's T lymphocytes genetically modified to express the Herpes Simplex Virus Thymidine Kinase (HSV-TK) as suicide gene. This allows the selective killing of dividing cells upon administration of the pro-drug ganciclovir (GCV), which is enzymatically phosphorylated to an active triphosphate analogue by HSV-TK. Triphosphate GCV competitively, inhibits incorporation of deoxyguanosine triphosphate (GTP) into elongating DNA, thus killing the proliferating cells.

If GvHD occurs, ganciclovir/valganciclovir will be administered. The activated, transduced T lymphocytes that are causing the GvHD should convert the GCV to its toxic form and thereby undergo apoptosis. This strategy allows the direct targeting of those T lymphocytes that are initiating the GvHD response. Relationships between dose and GvHD are discussed under Clinical safety and relationships between CD3+ and LNGFR+ peak concentrations and PFS, OS and NMR are discussed in the efficacy section of this report.

Overall, in the clinical study TK007, the 30 treated patients received their first infusion of Zalmoxis cells at a median time of 43 days from the date of HSCT. The median interval time between the first and the subsequent infusions of Zalmoxis cells was 30 days.

Immune-reconstituted patients reached a CD3+ cell count ≥ 100/µL at a median of 77 days after HSCT. In particular, at immune reconstitution Zalmoxis represents a high proportion of the circulating lymphocytes, while at later time points the proportion of Zalmoxis progressively decreases and untransduced lymphocytes expand from donor-derived precursors. One year post-Zalmoxis

administration the newly reconstituted T cell repertoire is dominated by untransduced cells of donor origin which displayed a polyclonal pattern comparable to healthy individuals.

2.4.5. Conclusions on clinical pharmacology

The nature and the intended use of the product are such that conventional studies on pharmacokinetics including absorption, distribution, metabolism and excretion are not applicable.

The pharmacological properties of Zalmoxis have been adequately studied within study TK007. Its primary mechanism of action relies on its ability to engraft and stimulate immune-reconstitution. If GvHD occurs, ganciclovir/valganciclovir will be administered; subsequently the activated, transduced T lymphocytes that are causing the GvHD should convert the GCV to its toxic form and thereby undergo apoptosis. This mechanism allows the direct targeting of those T lymphocytes that are initiating the GvHD response.

The product development changed over time and a different retroviral vector encoding for HV-TK gene was used. Upon analysis of the data of 29 samples, there are no indications that the differences in production process of the grafts impacted the likelihood to achieve IR. Therefore, there are currently no concerns on the impact of such changes on the clinical data from both TK007 and TK008.

The CHMP endorse the CAT assessment regarding the conclusions on the Clinical pharmacology as described above.

2.5. Clinical efficacy

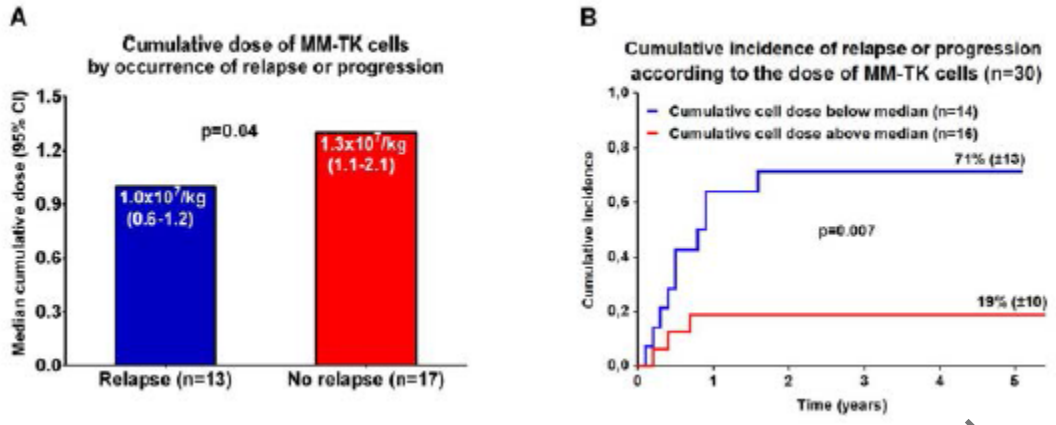
2.5.1. Dose response study(ies)

No formal dose response studies were conducted. The phase I/II study TK007 did contain a dose-response component with two doses transplanted, i.e. $1x10^6$ cells/kg or $1x10^7$ cells/kg IV.

The dose of infused cells did not impact on the percentage of patients that achieved IR: among patients starting with $1x10^6$ or $1x10^7$ cells/kg, there was no difference in proportion of IR (9 of 12; 75% versus 14 of 18; 78%, respectively) and proportion of IR after a single dose (5 of 9; 56% versus 8 of 14; 57%, respectively). No relevant differences were detected in results of analysis based on the first dose administered ($1x10^6$ or $1x10^7$ cells/kg) for time to first infusion, time to IR from last infusion, peak absolute count (Cmax) and time to reach the peak (Tmax) of CD3+ and LNGFR+ cells, Cmax of CD4+ and CD8+ counts.

There was also no relation between cumulative cell dose and IR, yet a relationship between the cumulative dose of MM-TK and an anti-leukaemia effect could be seen (see Figure 8). Based on this observation the recommended dose in TK008 is $1x10^7$ cells/kg of MM-TK. To what extent the higher cell dose in TK008 contributes to the apparent better results in TK008 is difficult to assess considering the additional differences between the two studies in characteristics of the MM-TK graft and of the patient population treated.

Figure 8: Relationship between cumulative dose of MM-TK cells and occurrence of relapse or progression



Based on the proposed specifications, up to 10% of the infused MM-TK cells are allowed to be negative for LNGFR, implying a lack of GCV responsiveness of near 1 x10^5 CD3+T-cells/kg that lack the HSV-TK gene. This is well above the threshold of 2x10^4 T-cells, below which occurrence of GvHD is very unlikely (Reisner Y., et al.). Thus, this higher dose of MM-TK may pose a safety risk, because of the higher dose of unmanipulated T-cells. Notably, this risk does not only depend on the cell dose, but also on the timing of infusion relative to HSCT. The more time between HSCT and T cell infusion, the lower the risk of GvHD. Therefore, at the time of MM-TK infusion, the threshold below which no GvHD may be expected is likely to be higher than mentioned above. Currently, the data indicate, that in the majority of GvHD cases, the transduced cells have been involved in the development of GvHD and that GCV treatment indeed has helped to resolve the GvHD (see also safety section). Also patients, in whom there was no proof of an effect of GCV on GvHD, were able to resolve the GvHD that was likely to be either caused by the unmanipulated T-cells in the MM-TK product, or by cells originating from the stem cell graft. So either way, the risk of GvHD caused by the presence of unmanipulated T-cells in the MM-TK product appears to be manageable. Therefore, further discussion about the dose is currently not required.

2.5.2. Main study

TK007

TK007 was a multicenter, international, open-label, non-randomized phase I-II study designed to evaluate the safety and activity of MM-TK cells in patients with haematological malignancies who underwent allogeneic HSCT from haploidentical donor.

Methods

Study Participants

Inclusion criteria

a. Patients ≥ 18 years old affected by haematological malignancies at high risk of relapse based on disease progression or presence of negative prognostic factors, who had received a HSCT from HLA-mismatched donor (haploidentical) for 2 or 3 loci

b. Engraftment documented by more than 500 neutrophils/μL for three consecutive days in the absence of growth factors

c. Mixed chimerism or full donor chimerism confirmed

d. Acute myeloid leukaemia (AML) in first or second relapse or primary refractory

e. High-risk AML in first or subsequent remission

f. Refractory anaemia with excess of blasts (RAEB) and RAEB in transformation

g. Chronic myeloid leukaemia (CML) in second chronic phase, blast crisis or accelerated phase

h. Poor prognosis acute lymphoblastic leukaemia (ALL) in first or subsequent remission

i. High grade Hodgkin disease (HD) or non-Hodgkin lymphoma (NHL) in third or subsequent remission

j. Multiple myeloma in advanced stage relapsing or progressing after high dose chemotherapy

k. Absence of fully HLA matched or one HLA locus mismatched family donor

l. Stable clinical conditions and life expectancy > 3 months

m. Performance status (PS) according to Karnofsky score > 70

n. Written donor/patient informed consent

Exclusion criteria

a. Infection with cytomegalovirus being treated with ganciclovir

b. Presence of GvHD grade > I requiring systemic immunosuppressive therapy

c. Ongoing systemic immunosuppressive therapy

d. Ongoing acyclovir administration

e. Administration after HSCT of granulocyte colony-stimulating factor (G-CSF) and cyclosporin A

f. CD3+ lymphocytes > 100/µl before infusion

g. Life-threatening condition or complication

h. CNS disease

i. Pregnant or lactating women

For the exclusion criteria a, b, c, d, and e, the MM-TK cells could however be administered after an adequate patient wash-out period.

Treatments

In absence of immune reconstitution (circulating CD3+ cell count ≥ 100/µL) and/or GvHD, the overall treatment plan consisted of up to four infusions given every 30 days at the following doses:

- first infusion: $1x10^6$ or $1x10^7$ cells/kg between day +21 and day +49 after HSCT
- second infusion: $1x10^7$ cells/kg
- third infusion: $1x10^6$ cells/kg plus interleukin-2 ($1x10^6$ IU/m2, subcutaneously, for 5 days)
- fourth infusion: $1x10^7$ cells/kg plus interleukin-2 ($1x10^6$ IU/m2, subcutaneously, for 5 days)

In patients receiving ganciclovir for cytomegalovirus infection, the infusion of MM-TK cells was given at least 24 hours after ganciclovir discontinuation.

In patients transplanted in relapse, investigators could start treatment with a dose different from that planned in the trial ($1x10^7$ cells/kg, instead of $1x10^6$ cells/kg).

For treating relapsed or progressive disease on study, protocol allowed, at investigator discretion, the administration of further MM-TK cells given as donor lymphocytes infusions (DLI) with or without further HSCT. In this case the dose was not fixed and, based on the results and patient clinical condition, the investigator requested a specific dosage of MM-TK cells. The DLI was given upon confirmation of disease relapse, during or after the follow-up phase.

Identity of investigational product: MM-TK cells

The drug product is defined as frozen haploidentical donor T lymphocytes genetically modified with the retroviral vector SFCMM-3 Mut2 #48 (SFCMM-3 Mut2 #48 transduced lymphocytes), encoding for the ΔLNGFR and HSV-TK Mut2 genes in the final formulation and container closure system, ready for intended medical use.

MM-TK is a patient-specific product prepared starting from a lymphocyte apheresis of a haploidentical donor. At least 20 days before HSCT, donor peripheral lymphocytes were collected at investigational sites from haploidentical donors, before mobilisation of haemopoietic precursors in order to avoid the immune-modulatory effect of G-CSF.

The lymphocyte apheresis bag was sent to MolMed GMP facility for manipulation.

Initially, the investigational product consisted of fresh HSV-TK cells, whereas a freezing step was subsequently introduced at the end of manufacturing process to obtain cryopreserved cells. This change in drug formulation was implemented for both logistic reasons and better efficiency assessment before infusion. For this reason, after completion of first and second study stage with 18 treated patients, an additional 12 patients were subsequently included to test biosimilarity between fresh and cryopreserved cells.

Ganciclovir for treatment of GvHD related to MM-TK cells

Ganciclovir (GCV) is the drug of choice for treatment and pre-emptive strategies for CMV infection in allogeneic HSCT and extensive experience has been accumulated with this drug.

The use of genetic engineering of donor lymphocytes with the herpes simplex virus-thymidine kinase (HSV-TK) suicide gene confers the ability to modulate GvHD by in vivo ganciclovir-induced elimination of the transduced cells. The HSV-TK proteins convert the pro-drug ganciclovir to its monophosphate intermediate derivate. Cellular kinase phosphorylates it to a triphosphate (GCV-3P) compound which is the toxic form. GCV-3P can be incorporated into DNA, replacing deoxyguanosine triphosphate, resulting in inhibition of DNA chain elongation and causing cell death.

Ganciclovir is an intravenously administered drug that requires hospitalization, and therefore it was sometimes replaced with the oral pro-drug valganciclovir.

Valganciclovir is absorbed and rapidly metabolized to GCV in the intestinal wall and liver. The bioavailability of ganciclovir from valganciclovir is approximately 60%, and the systemic exposure to valganciclovir 1800 mg daily per os provides comparable systemic exposure to that of 10 mg/kg daily per intravenous infusion of ganciclovir.

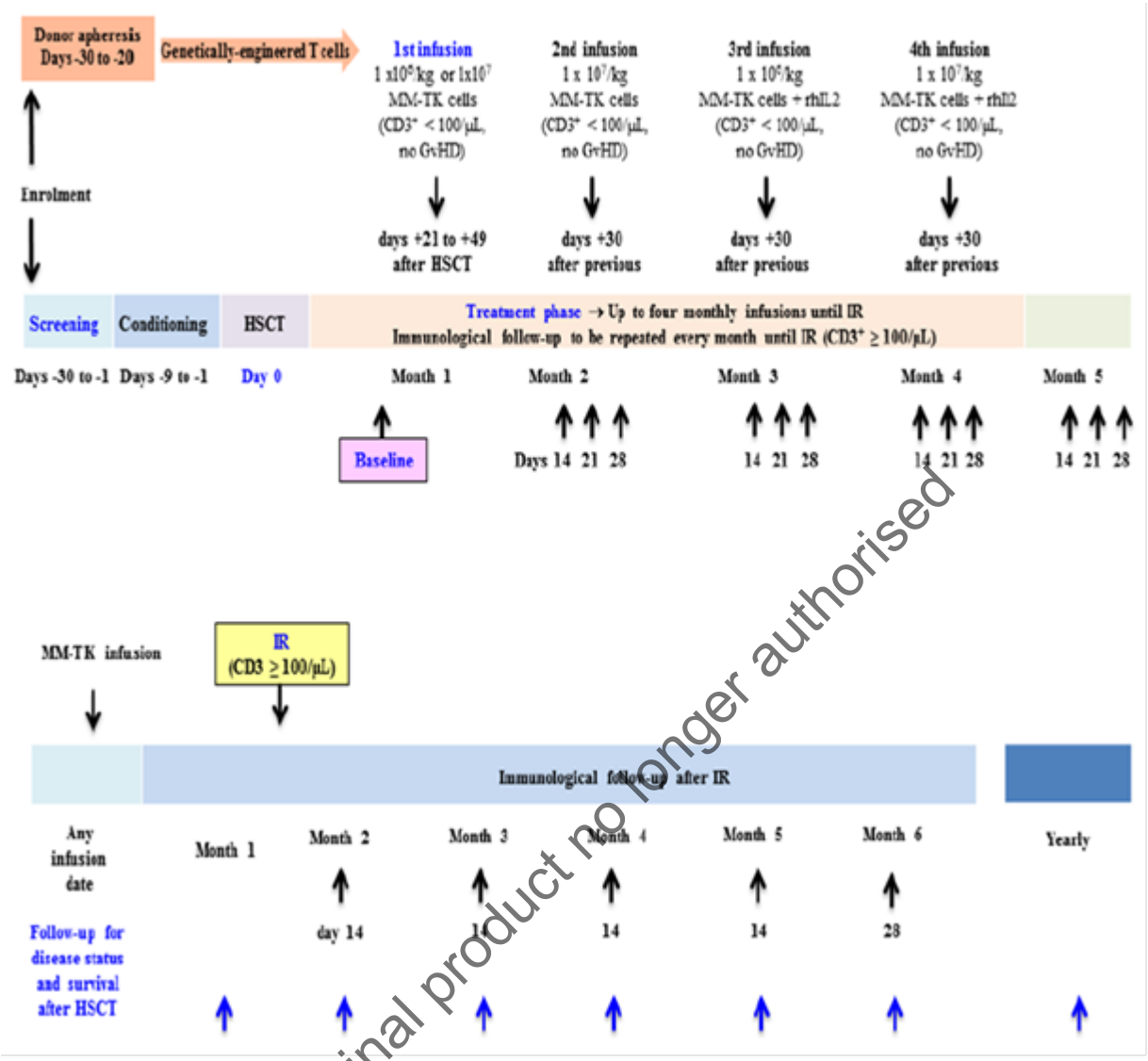
In case of grade ≥ 2 acute or chronic GvHD related to MM-TK cells, the recommended treatment was ganciclovir 5 mg/kg/day or valganciclovir 900 mg two times per day for 14 days.

Interleukin-2 in combination with MM-TK cells (third and fourth infusion)

The recombinant interleukin-2 at dosage of $1x10^6$ IU/m^2 subcutaneously administered for five days was planned in combination with the third and the fourth dose of MM-TK cells infusions to increase the differentiation and proliferation of T cells and antitumor effects.

The overall procedures of the study are described in Figure 9.

Figure 9: Overall study Procedures (study TK007)



Objectives

The primary objectives were the evaluation of clinical activity in terms of immune reconstitution (IR) after haploidentical HSCT, the evaluation of the in vivo control of GvHD after administration of ganciclovir in patients treated with HSV-TK transduced T cell and the evaluation of graft-versus-leukemia (GvL) effect.

Secondary objectives were the evaluation of disease free survival and overall survival, the evaluation of incidence of infectious events and the evaluation of acute and long-term toxicity related to the infusions.

Outcomes/endpoints

The primary endpoint was the proportion of patients who achieved immune reconstitution (IR), empirically defined a priori as an absolute CD3+ cell count of 100/µL or more for two consecutive observations (and/or CD4+ cells ≥ 50/µL and/or CD8+ cells ≥ 50/µL).

Secondary endpoints were the following:

Time to IR :defined as the time to reach an absolute CD3+ cell count ⩾ 100/ µL for two consecutive

observations (and/or CD4+ cells ⩾ 50/μL and a CD8+ cells ⩾ 50/μL), starting from the first date of HSCT (and, additionally, from the date of the first and last infusion).

Cumulative incidence of grade 2 to 4 acute GvHD (aGvHD): diagnosed and graded according to standard criteria and computed from the date of HSCT. Death without occurrence of aGvHD was considered competing event.

Cumulative incidence of extensive chronic GvHD (cGvHD): diagnosed and graded according to standard criteria and computed from the date of HSCT for patients surviving at least 100 days. Death without occurrence of cGvHD was considered competing event.

Disease/progression-free survival (DFS/PFS): measured from the date of HSCT until the date of disease relapse (for patients in complete remission at HSCT) or disease progression (for patients with active disease at HSCT) or death from any cause, whichever occurs first. The term progression refers to any stage advanced of that at the date of HSCT.

Overall survival (OS): measured from the date of HSCT until death resulting from any cause.

Cumulative incidence of non-relapse mortality (NRM): defined as any death not preceded by disease relapse/progression, which was considered as competing event. Patients alive without disease relapse/progression were censored at last date known to be alive.

Cumulative incidence of relapse/progression: based on hematologic, morphologic, cytogenetic, or radiologic evidence of relapse or progression, as appropriate.

Acute and long-term toxicity related to MM-TK cells, with adverse events (AEs) graded according to National Cancer Institute Common Toxicity Criteria (NCI-CTC version 3.0).

Sample size

This phase I/II TK007 trial was designed according to Simon's two-stage design method. The method provides the number of patients to enrol in the first (n1) and second stage (n) of the study. To apply this method one must specify: a target rate of interest (P1), a rate of no interest (P0), the levels of α (type 1 error) and β (type 2 error). The table below shows the calculation of n, n1, r1, r2 after fixing P0 (rate of immunereconstitution under the hypotesis that the therapy is not efficacious) equal to 0.15, P1 to 0.5 (rate of immune-reconstitution under the hypotesis that the therapy is efficacious) and α and β to 0.05 and 0.10, respectively.

Optimum design (α=0.05, β=0.10)

P_0	P_1	n	r_1/n_1	r_2/n	PET_P_0	PET_P_1	ANP
0.15	**0.5**	**18**	**1/7**	**5/18**	**0.72**	**0.6**	**10**

Simon's method suggested that 7 patients have to be enrolled in the first phase. If the number of responses is equal or less than 1 the study will terminate at this stage. Under the hypothesis that P0 is the true rate such a result has a probability of 0.72 to occur. If r1 is greater than 1 then the enrollment will continue until the end of the second stage. The second stage ends when the 18th patient has been enrolled. The therapy will be considered a success if the number of responders is equal or greater than 6.

Additionally, 12 patients were included to test biosimilarity between fresh and cryopreserved cells.

Randomisation

This was a single arm study.

Blinding (masking)

This was an open-label study.

Statistical methods

Definition of study populations

The ITT population included all enrolled patients who received at least one haploidentical HSCT. Two additional patient populations were identified:

- the standard (or treated-patient) population, which included all patients who received at least one infusion of MM-TK cells, and
- the subset of treated-patient population who achieved IR as previously defined.

Concerning the effect analyses in pivotal study TK007

The first date of HSCT (study day 0) was the starting point for calculating all the time-to-event outcomes. For patients undergoing more than one transplantation from the same donor, outcomes were computed from first HSCT, while for patients undergoing transplantation from different donors, the outcomes were computed from last HSCT.

Continuous variables were summarized by mean with standard deviation (SD) or median with 95% confidence interval (95% CI), interquartile range (IQR; 25^{th} to 75^{th} percentile) and range (minimum to maximum). Categorical variables were summarized by frequency and proportion of patients in each category. The 95% CI of proportions were computed using a modified Wald method.

Comparisons of categorical data were done with the Pearson chi-squared test or Fisher exact test as applicable, while unpaired and paired continuous data were compared using Mann-Whitney test and Wilcoxon test, respectively. For handling missing data, a complete-case analysis was used by excluding patients with missing values from the data set.

Rates of NRM, relapse/progression, acute GvHD (aGvHD) and chronic (cGvHD), with related standard error (SE), were estimated using the cumulative incidence function, to adjust analysis for presence of competing events. The Gray test was used to compare cumulative incidences of competing-risk endpoints (Gray, 1988).

Median times for DFS/PFS and OS were estimated using the product-limit Kaplan-Meier method and the 95% CI was calculated with the Brookmeyer and Crowley method. The log-rank test was used for univariate comparisons of survival function between groups with different covariates and the hazard ratio (HR), with related 95% CI, was computed using a Cox proportional-hazards model. Follow-up duration was calculated according to the inverse Kaplan-Meier approach (Shemper et al, 1996).

Univariate and multivariate logistic and Cox regression models were fitted to test associations of the outcomes of DFS/PFS and OS with patient, donor and disease-related variables available at study screening:

- patient age (equal or greater than median versus lower than median);
- patient sex (male versus female);
- patient performance status (equal or greater than 90 versus lower than 90);
- time from diagnosis to HSCT (equal or greater than 12 months versus lower than 12 months);
- donor/patient sex combination (female/male versus other combinations);
- donor/patient Natural Killer (NK) alloreactivity (yes versus no);

- diagnosis (acute myeloid leukaemia (AML) versus other diagnoses);
- disease status at HSCT (complete remission versus relapse).

To mitigate time-guarantee bias, univariate and multivariate Cox proportional hazards methods, in which administration of MM-TK cells and achievement of IR were modelled as time-dependent covariates, were used to estimate unadjusted and adjusted hazards ratios for MM-TK treatment and IR effects on DFS/PFS and OS. In time-dependent covariate analysis, coding of treatment and IR were changed from absent to present at the time of their first occurrence.

Statistical analyses were done using SAS (version 9.2). All the reported p values were two-sided and were considered significant if equal or less than 0.05, without formal multiplicity adjustments.

Concerning the comparison of the TK007 results with historical controls

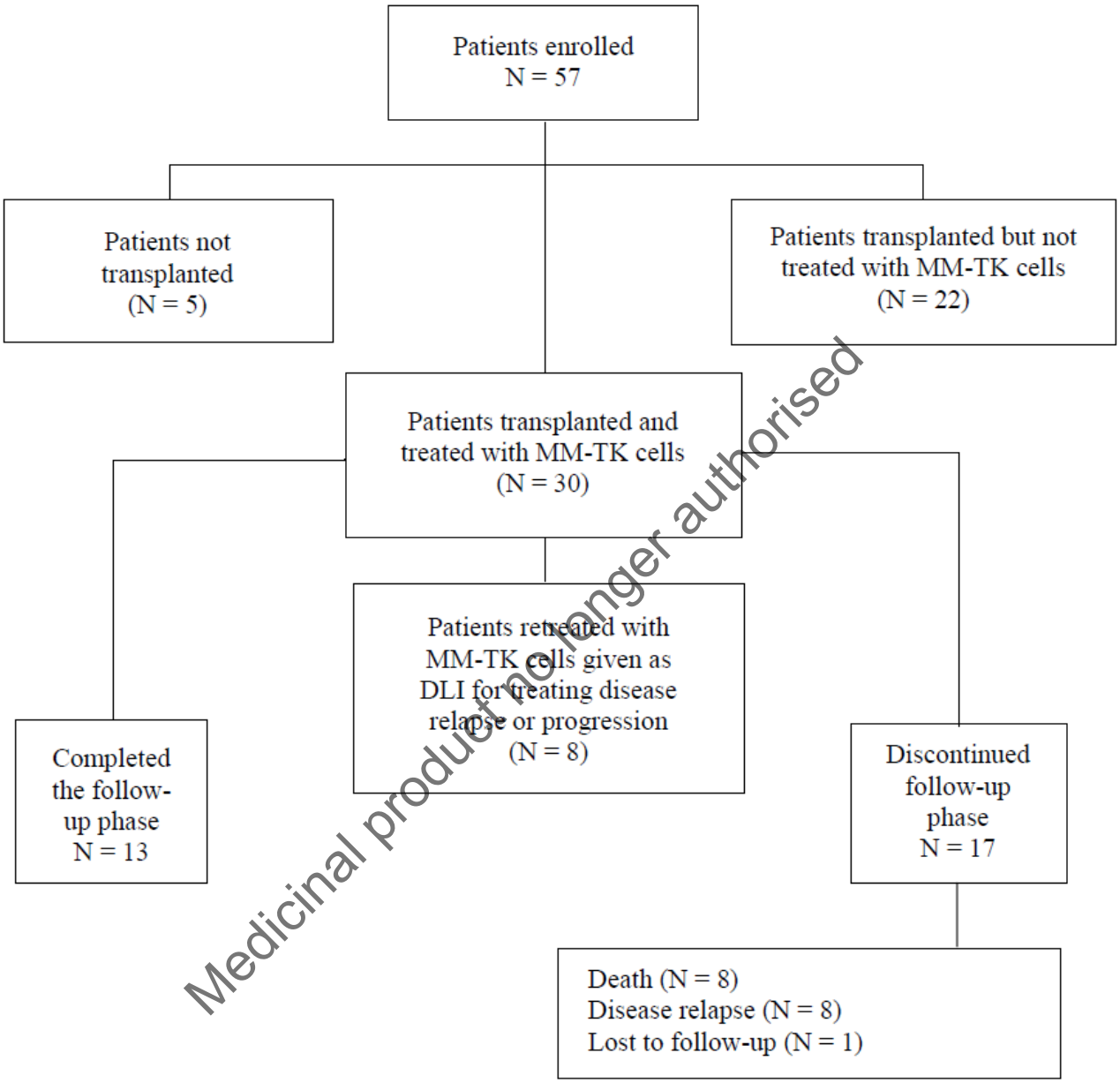
Currently, there are neither approved therapy nor widely accepted standard of care able to overcome the two intertwined problems that continue to account for most of the non-relapse deaths, opportunistic infections and GVHD, as well as to improve relapse-free rates after haploidentical HSCT.

Therefore, the magnitude of treatment effect and clinical benefit of MM-TK cells can be only assessed versus historical control data from either a large retrospective survey (Ciceri et al, 2008) or single-centre experiences (Aversa et al, 2005; Mehta et al, 2004; Luznik et al, 2008; Munchel et al, 2011) that have been reported in similar patient populations undergoing haploidentical HSCT.

Additional comparisons with historical control data from patients who are included in EBMT data base for the two treatment options (T-cell depleted and T-cell replete, with or without add back strategies), with a pre-specified search strategy, endpoints and statistical methods for comparing the historical controls to the uncontrolled TK007 Phase II results.

Results

Participant flow TK007



Recruitment

The study centres were located in Milan (IT), Rozzano (IT), Perugia (IT), Pescara (IT), London (UK), Hannover (DE), Thessaloniki (GR) and Jerusalem (IL).

Conduct of the study

The enrolment phase was 6 years and the follow-period lasted 5 years. The table below lists changes implemented in the clinical protocol.

Table 4: Summary of protocol amendments

Protocol Amendment	Date	Summary of amendment objectives
Version B	October 23rd, 2002	Inclusion of additional sample collection for LNGFR$^+$ and PCR-TK at the 3rd and 5th month time points during the follow-up phase. Collection of buffy coat at screening phase for immunological studies.
Version C	April 24th, 2003	Changes in the time points of sample collection for immuno phenotype analysis: from every-week to every-2-week schedule during the first month and from every-2-week to every-4-week schedule during the second and third month. Introduction of ISHAGE method for CD3$^+$, CD4$^+$ and CD8$^+$ assessment Inclusion of specific procedures to be followed in case of potential pregnancy during study (protocol section 11.1) Use of DLI for disease relapse extended after the 6th month of follow-up, thorough the study
Version D	September 1st, 2003	Definition of safety data collection in case of DLI during the entire study (protocol section 11.1) and introduction of follow-up phase for patients undergoing DLI for disease relapse (protocol section 9.7)
Version E	March 1st, 2005	Addition of further reduced toxicity conditioning regimens for a patient subset affected by lymphoid or myeloid haematological disease, with age > 55 years and/or presenting with associated comorbidity or organ impairment (protocol section 7.3.4)
Version F	May 31st, 2005	Introduction of a freezing step the end of manufacturing process of the MM-TK cells. In order to assess the therapeutic equivalence between fresh cells and cryopreserved cells, an additional cohort of 3 to 6 patients was included after the enrolment of the 18th evaluable patient (protocol sections 2.3, 10.1 and 12.1)
Version G	June 14th, 2007	Inclusion of an additional cohort of 6 patients to be treated with cryopreserved cells. Enlargement of the interval timing and anticipation of the first infusion of MM-TK cells after HSCT at 21 to 49 days (instead of 35 to 49 days) (protocol section 6.1). Investigator choice for a starting dose of 1x10^6 or 1x10^7 cells/kg applied for all patients (protocol section 6.1).

Baseline data

Table 22 summarizes patient, donor and disease characteristics at screening phase for the 52 transplanted patients, including 30 patients who received MM-TK cells and 22 patients who did not. Median patient age was 49 years, with 12 patients (23%) being 60 years or older, 26 patients (50%) having de novo AML, 10 with secondary AML (19%) and 31 patients (60%) complete remission at HSCT. The median number of prior treatment lines was 2 (range, 0 to 5).

Table 5: Patient / donor and disease characteristics at screening

Variable	All patients n=52 (%)	Untreated patients n=22 (%)	Treated patients n=30 (%)
Patient age			
Median in years	49	49	49
Range	17 - 66	18 - 65	17 - 66
25^{th} - 75^{th} percentile	35 - 57	41 - 56	32 - 57
≥ 60 years	12 (23)	5 (23)	7 (23)
Gender			
Male	22 (42)	12 (55)	10 (33)
Female	30 (58)	10 (45)	20 (67)
Karnofsky performance status			
100	37 (71)	14 (64)	23 (77)
90	3 (6)	1 (4)	2 (7)
80	8 (15)	3 (14)	5 (17)
70	3 (6)	3 (14)	-
Not available	1 (2)	1 (4)	-
Time from diagnosis to HSCT			
Median in months	10.6	11.3	9.8
≥ 12 months	22 (42)	10 (45)	12 (40)
< 12 months	30 (58)	12 (55)	18 (60)
Diagnosis			
AML	26 (50)	11 (50)	15 (50)
Secondary AML	10 (19)	3 (14)	7 (24)
MDS/RAEB/RAEB-T	7 (13)	3 (14)	4 (13)
HD/NHL	4 (8)	3 (14)	1 (3)
ALL	3 (6)	-	3 (10)
CML	1 (2)	1 (4)	-
Biphenotipic leukemia	1 (2)	1 (4)	-
Complete remission at HSCT	31 (60)	11 (50)	20 (67)
First	16	4	12
Second	11	4	7
Third	4	3	1
Relapsed/progressive disease at HSCT	21 (40)	11 (50)	10 (33)
Donor age			
Median in years	41	46	36
Donor gender			
Male	33	15	18
Female	19	7	12
Donor/patient gender			
Male/male	13	7	6
Male/female	20	8	12
Female/female	10	2	8
Female/male	9	5	4
Donor/patient CMV serostatus			
Positive/positive	39 (75)	15 (68)	24 (80)
Positive/negative	-	-	-
Negative/negative	4 (8)	3 (14)	1 (3)
Negative/positive	7 (13)	3 (14)	4 (13)
Not available	2 (4)	1 (4)	1 (3)
Donor/patient EBV serostatus			
Positive/positive	45 (86)	18 (82)	27 (90)
Positive/negative	2 (4)	1 (4)	1 (3)
Negative/negative	-	-	-
Negative/positive	1 (2)	1 (4)	-
Not available	4 (8)	2 (9)	2 (7)
Donor/patient NK alloreactivity			
Yes	23 (44)	9 (41)	14 (47)
No	27 (52)	13 (59)	14 (47)
Unknown	2 (4)	-	2 (6)

Numbers analysed

Table 6: Main data set analysed in the study

Populations	Number of patients
Patient enrolled	57
Patients not included in the intent-to-treat (ITT) population	5
Patients included in the ITT population	52
Patients not included in the treated-patient population	22
Patients included in the treated-patient population	30
Patients treated who did not achieve immune reconstitution	7
Patients treated who achieved immune reconstitution	23
Patients included in the safety population	52

Outcomes and estimation

IR as assessed by circulating T lymphocytes (CD3+ and/or CD4+ and/or CD8+).

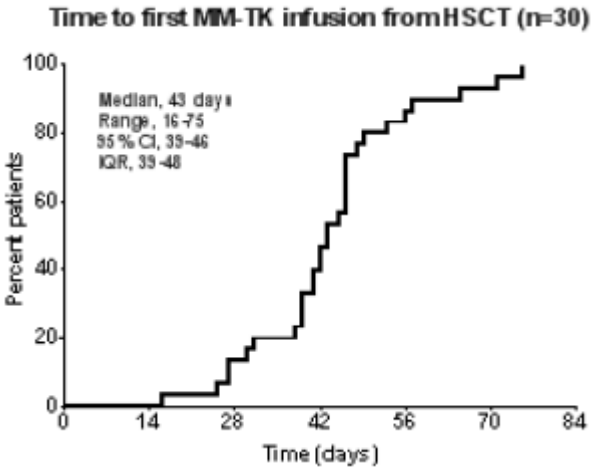
Overall, 23 of 30 MM-TK treated patients (77%; 95% CI, 59 to 88) reached the protocol-defined IR. This percentage of IR seems to indicate the feasibility of the treatment in particular considering the fact that a median of 1 infusion was required to establish IR with a median cumulative dose of $1x10^7$ MM-TK cells/kg. It is, furthermore, reassuring that baseline characteristics were equally distributed between MM-TK treated patients that reached IR and those that did not. Also there was no difference in starting dose ($1x10^6$/kg or $1x10^7$ cells/kg), cumulative cell dose (median, $1.3x10^7$/kg versus $1.0x10^7$ cells/kg), number of infusions (median, one for both) or fresh vs frozen MM-TK cells between the IR (n=23) vs the non-IR patient group (n=7), respectively.

Only three patients (TK3-11-52) received the third and the fourth MM-TK infusion and only one patient (TK11) received IL-2, which was associated with toxicity. None of the three patients who received the third and the fourth MM-TK dose reached immune reconstitution. Two additional patients received two non-protocol-mandated IL-2 courses. The impact of adding IL-2 after the first two infusions of MM-TK cells was deemed negligible and, therefore, IL-2 was not included in the treatment plan of the phase III trial TK008.

Time to IR

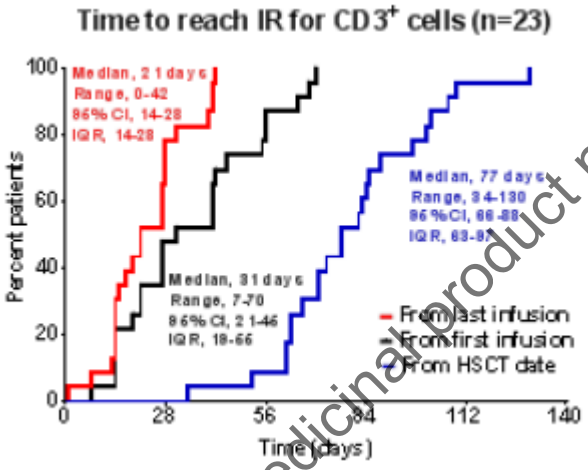
Treated patients received their first infusion of MM-TK cells at a median of 43 days from the date of HSCT (Figure 10), without difference detected between patients who achieved IR (n=23; median, 45 days; 95% CI, 41 to 48) and patients who failed to attain IR (n=7; median, 38 days; 95% CI, 27 to 53). The median interval time between the first and the subsequent infusions of MM-TK cells was 30 days (range, 27 to 39; 95% CI, 28 to 38).

Figure 10: Time to first infusion of MM-TK cells



Immune-reconstituted patients reached a CD3+ cell count ≥ 100/µL after a median of 77 days (95% CI, 66 to 88) from the date of HSCT, 31 days (95% CI, 21 to 45) from the date of first infusion and 21 days (95% CI, 14 to 28) from the date of last infusion of MM-TK cells), with CD4+ cells tending to recover slightly slower than CD8+ cells (data not shown).

Figure 11: Time to immune reconstitution (IR) for CD3+cell counts



Furthermore, the timing to both first dose of MM-TK cells and IR reconstitution was not different among patients receiving myeloablative or reduced-intensity preparative regimens, fresh or cryopreserved MM-TK cells and a first dose of $1x10^6$ or $1x10^7$ cells/kg. The time to CD3+ peak was not influenced by starting dose, but was quicker in case the MM-TK cells were frozen as compared to being fresh cells.

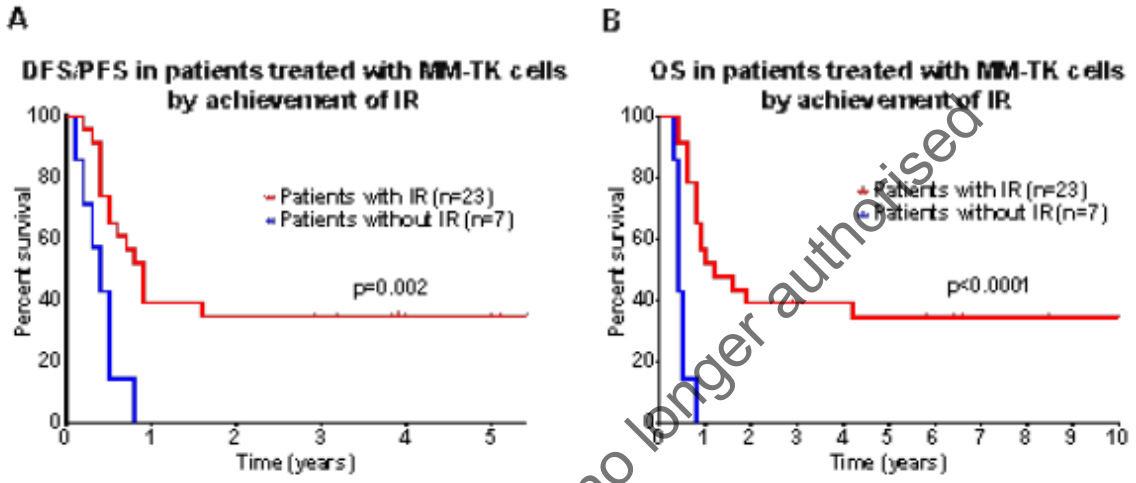
According to the protocol, MM-TK infusion was planned between day 21 and 49 post-HSCT in the absence of IR or GvHD. However, treated patients received their first infusion of MM-TK cells at a median of 43 days from the date of HSCT ranging from 16 days to 75 days post-HSCT. The delay in administration was caused by decisions from the treating physician, and were in part related to the inclusion criteria (e.g. delay for CMV infection requiring GCV treatment). The consequence of these protocol deviations is that heterogeneity regarding the baseline parameters is likely to be larger than planned. It is difficult to ascertain the impact of this on the study conduct and results, though considering the explanations provided, it is assumed to be limited.

Disease/progression-free survival (DFS/PFS)

The Applicant presented DFS/PFS data according to patients treated with MM-TK (N=30) vs patients untreated with MM-TK (n=22) and patients with IR (n=23) vs patients without IR (n=29).

Upon comparison of MM-TK treated patients with and without IR (Figure 12), it is clear that IR upon administration of MM-TK cells is associated with increased OS, fewer infectious events and reduced NRM. The effect of IR on relapse cannot be adequately analysed, because as indicated by the Applicant, NRM and relapse are competing risk events. Patients without IR tended to die before relapse could occur. Overall, the results are suggestive of IR being an early surrogate marker for efficacy in this small uncontrolled study in terms of OS, infections and reduced NRM.

Figure 12: DFS/PFS and OS according to IR in patients treated with MM-TK cells (n=30)



Overall survival (OS)

The Applicant presented OS data according to patients treated with MM-TK (N=30) vs patients untreated with MM-TK (n=22) and patients with IR (n=23) vs patients without IR (n=29).

Similar as with the DFS/PFS results, the presented OS data by treatment with MM-TK cells (n=30) represent the effect of combining HSCT and the administration of MM-TK cells, resulting in a OS at 1 year of 40%, at 2 years of 30% and a 5 year OS of 27%.

To further address the role of IR upon MM-TK cell administration as a surrogate for long-term outcome also in patients who underwent HSCT in complete remission (CR), the rates of the main endpoints (DFS, OS, infections, NRM and relapse) of the 15 patients with CR at HSCT who achieved IR were compared with those of the 5 patients with CR at HSCT who did not achieve IR. In comparison with patients without IR, those with IR had a 75% lower risk of relapse or death (unstratified HR=0.25 for DFS) and an 86% lower risk of death (unstratified HR=0.14 for OS). Thus, also in this population, IR is associated with increased OS, fewer infectious events and a reduced NRM, but the patient numbers involved are small.

Non-relapse mortality (NRM)

By ITT-population analysis (n=52), the cumulative incidence of non-relapse mortality was 50% (±7%) both at 1 year and 5 years. The cumulative incidence of non-relapse mortality was 17% for the 23 immune-reconstituted patients, with 4% (±4) because of infection, and 76% for the 29 non-immune-reconstituted patients, with 38% (±9) because of infection.

Table 7: Cumulative incidence rates of non-relapse mortality (NRM)

Cumulative incidence of NRM, % (±SE)						
Patients	**n**	**100 days**	**6 months**	**1 year**	**5 years**	**p value**
All	52	27 (±9)	40 (±8)	50 (±7)	50 (±7)	-
Untreated with MM-TK cells	22	64 (±10)	73 (±9)	77 (±9)	77 (±9)	<0.0001
Treated with MM-TK cells	30	0	17 (±7)	30 (±8)	30 (±8)	
Non immune reconstituted	29	48 (±9)	66 (±9)	76 (±8)	76 (±8)	<0.0001
Immune reconstituted	23	0	9 (±6)	17 (±8)	17 (±8)	

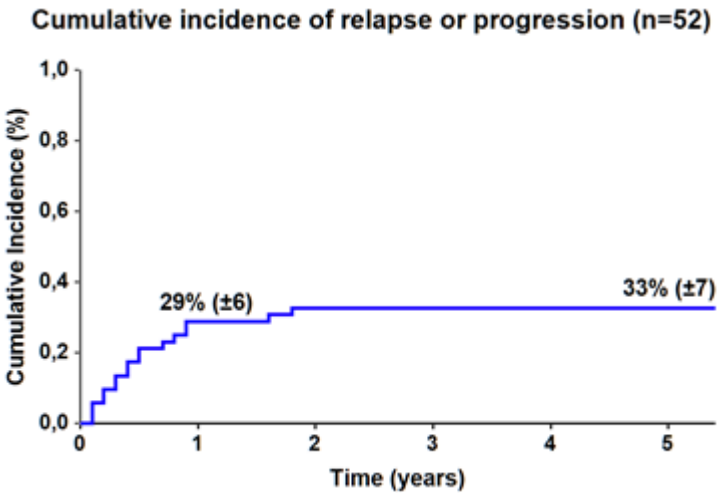
The data indicate that the patients treated with MM-TK have a lower NRM as compared to the patients that were not treated. However, the results from this comparison are difficult to interpret as the patients who can actually be treated with MM-TK are those patients that had not died and had myeloid engraftment, no GvHD, though no IR, by the time that infusion with MM-TK cells would become at the patient's disposal. This means that the remaining patients represent a selected population that is likely to have a better prognosis anyway as compared to the patients that lost eligibility for the MM-TK cells after HSCT. A similar argumentation can be applied for the comparison in terms of NRM (due to infection) in the IR vs the non-IR patients.

In order to determine the effect of the combination of the HSCT and the infusion of the MM-TK cells, the results from all MM-TK treated patients (n=30) should be compared to the NRM as observed in historical controls. As stated earlier, the details of the comparison of the MM-TK data with historical control group from the EBMT database, such as the strategy for the matched-pair analyses, the patient characteristics and outcomes will be discussed at the end of this section.

Relapse incidence (RI) (as a measure for GvL effect)

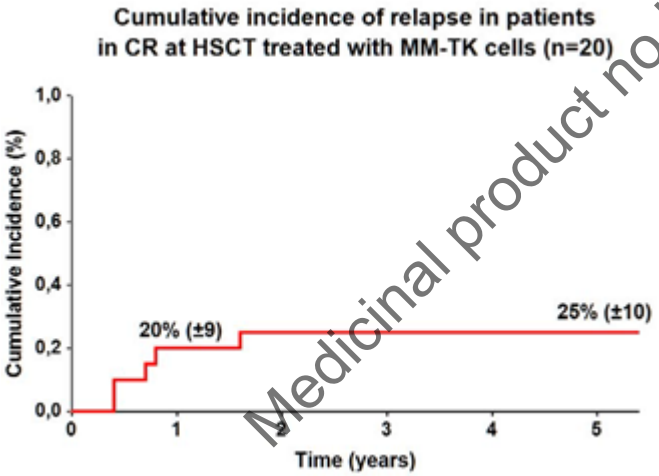
The cumulative incidence of disease relapse or progression for all transplanted patients (n=52) was 29% at 1 year and 33% at 5 years (Figure 13), while the cumulative incidence of progression at 1 year was 48% for patients transplanted with active disease (n=21) and at 5 years 23% for those transplanted in remission (n=31).

Figure 13: Cumulative incidence of disease relapse or progression for all transplanted patients.



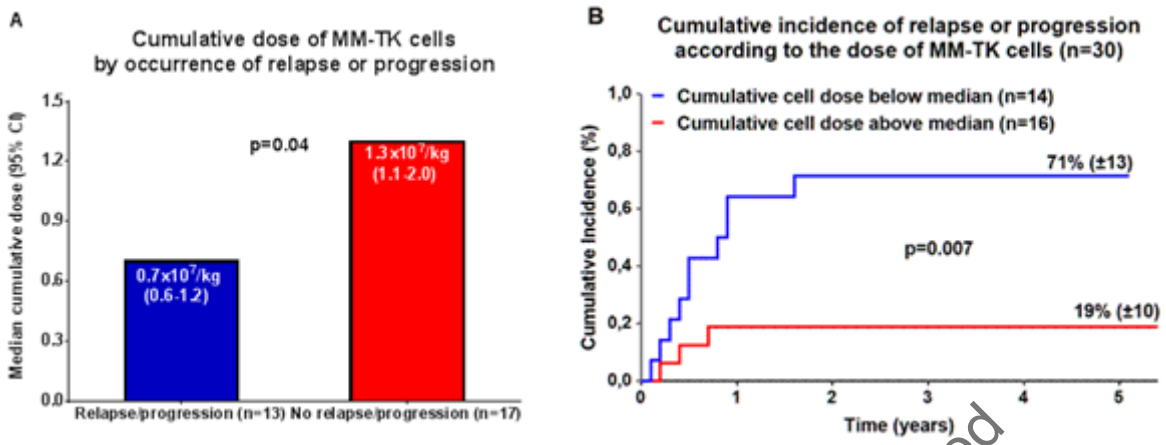
With respect to MM-TK treatment, among patients transplanted in remission (Figure 14), the cumulative incidence of relapse at 5 years was 25% (±10) for those who received MM-TK cells (n=20). For comparison, the cumulative incidence of relapse at 5 years was 33% (±13) for those in CR at HSCT and who achieved IR upon MM-TK (n=15).

Figure 14: Cumulative incidence of relapse for patients in complete remission (CR) at HSCT receiving MM-TK cells



For the overall MM-TK treated population (n=30), there was no difference between the patients with or without IR regarding the median cumulative cell dose, i.e. $1.3x10^7$ versus $1.0x10^7$ cells/kg. Compared with patients who relapsed or progressed, those who did not experience disease relapse or progression had received significantly higher cumulative doses MM-TK cells ($1.3x10^7$ cells/kg vs $0.7x10^7$ cells/kg; p=0.04) (Figure 15A). Accordingly, patients who had received doses of MM-TK cells greater than median ($1.1x10^7$ cells/kg), experienced a cumulative incidence of relapse or progression of 19%, as compared with 71% reported for patients who had received cell doses less than median (p=0.007) (Figure 15B).

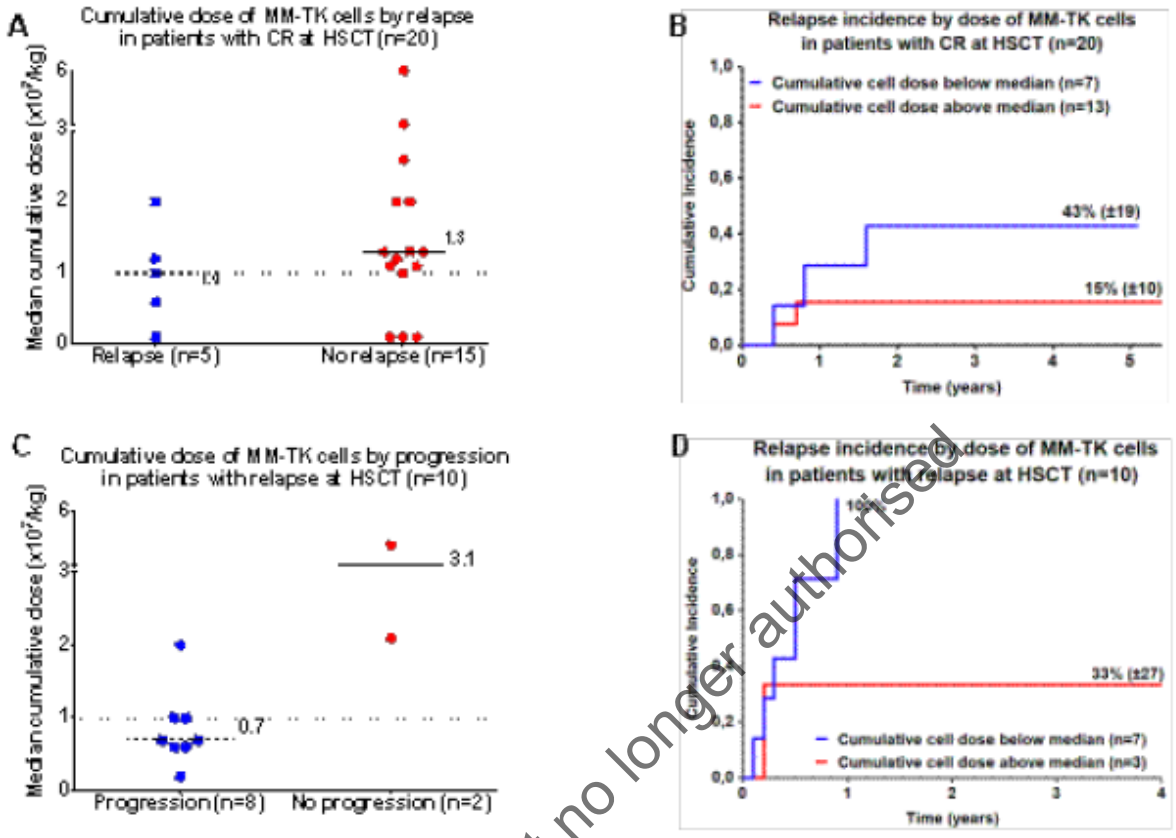
Figure 14: Relationship between cumulative cell dose of MM-TK cells and occurrence of relapse or progression



The relationships between cumulative dose of MM-TK cells (median, $1.1x10^7$/kg) and occurrence of relapse were assessed for the 20 patients in CR at HSCT. Compared with the five patients who relapsed after HSCT, the 15 patients who did not relapse had received a little greater dose (median, $1.3x10^7$/kg versus $1.0x10^7$/kg; p=0.29) (Figure 16A) and more frequently a dose higher than $1.0x10^7$/kg (11 of 15 patients (73%) versus 2 of 5 patients (40%)). The relapse incidence in patients who had received doses above the median was two-third lower than in those who had received doses below the median (15% versus 43%; p=0.21) (Figure 16B).

Similar relationships between dose and relapse were noted for the 10 cases in relapse at HSCT. Compared with the eight patients who relapsed after HSCT, the two patients who did not relapse had received a greater dose (median, $3.1x10^7$/kg versus $0.7x10^7$/kg; p=0.06; figure 16C). Thus, the relapse incidence in patients who had received doses above the median resulted two-thirds lower than in those who had received doses below the median (33% versus 100%; p=0.13; figure 16D).

Figure 16: Relationships between cumulative dose of MM-TK cells and occurrence of relapse on study according to the disease status at HSCT



While patient numbers are limited, the data suggest that a higher dose of MM-TK cells is associated with a reduced relapse rate, both in the patients with CR at time of HSCT (N=20), as those without CR (n=10).

Infectious events

Over a median observation time of 3.8 months (range, 0.1 to 13.4) (which was computed from the HSCT to the date of resolution of the last infection recorded), a total of 249 infectious AEs were reported for 47 patients. For five patients (TK2-7-19-49-56) no infections were registered. Overall, the median number per patient of infectious AEs was 5 (range, 1 to 20; 95% CI, 4 to 6), with a median duration per event of 11 days (range, 1 to 84).

The Applicant provided results showing that the median number per patient and per month of infectious AEs was 1.5 (95% CI, 1.2 to 1.8) and, in comparison with non-immune-reconstituted patients (n=24), it was significantly reduced in immune-reconstituted patients (n=23) and in patients who developed GvHD (n=11), remaining in these patients as low as in immune-reconstituted patients who did not develop GvHD (n=12)

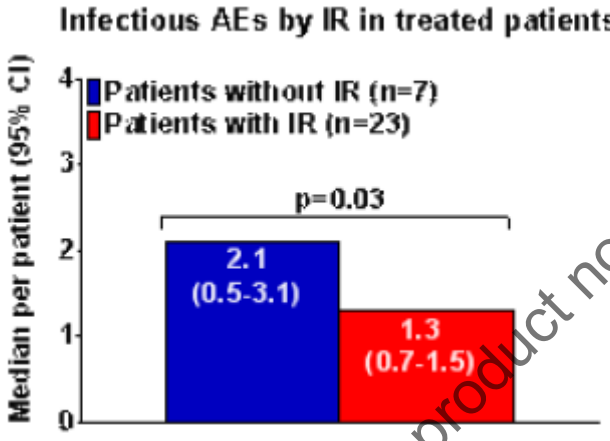
Regarding the relation between GvHD and infectious events, it is acknowledged that there does not appear to be a correlation between the presence or absence of GvHD and the median number of infectious events per patient.

The Applicant also described the occurrence of viral infections that accounted for 45% (112 of 249) of all infectious AEs and occurred in 36 patients (median per patient, 3; range, 1 to 7), with a median duration per event of 15 days (range, 1 to 84). The median time to developing viral infections in

relation to HSCT was 69 days (range, 3 to 237). Among patients treated with MM-TK cells, 58 events occurred in 21 immune-reconstituted patients and 19 in five non-immune-reconstituted patients, whereas 10 events developed in six untreated patients. Compared with non-immune-reconstituted patients, there was a trend in reductions in frequency and length of these events, as well as duration of foscavir and/or ganciclovir treatment in immune-reconstituted patients (data not shown). Also a total of 12 (5%) Epstein-Barr virus (EBV) infections were recorded in 10 patients (median, 1; median duration, 17 days). Among patients treated with MM-TK cells, eight EBV infections occurred in seven patients with IR and four in three patients without IR, while no event developed in untreated patients. Among patients who achieved immune reconstitution, both frequency and duration of viral infections were not different between patients who developed GvHD (n=11; median number: 4; median duration, 15 days) and patients who did not suffer GvHD (n=12; median number, 3; median duration, 18 days).

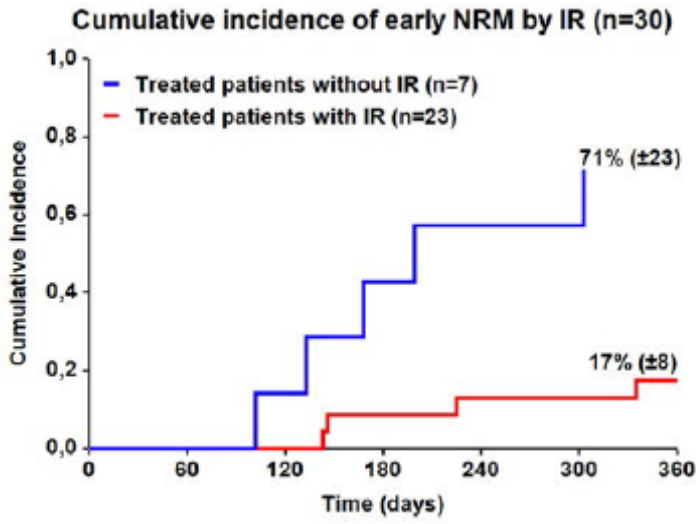
Additional analyses showed that the median number per patient and per month of infectious adverse events (AEs) among patients treated with MM-TK cells was lower in those who reached IR (n=23) than in those who did not reach IR (n=7) (Figure 17).

Figure 17: Incidence rates of infectious AEs according to IR in patients treated with MM-TK cells (n=30)



This reduction in the infectious AEs was associated to a substantial reduction of the 1-year NRM, which was 17% for the 23 patients with IR and 71% for the 7 patients without IR (see Figure 16), with five non-immune-reconstituted patients rapidly dying 3 to 10 months after HSCT, without evidence of prior occurrence of relapse or progression.

Figure 18: Early NRM according to IR in patients treated with MM-TK cells (n=30)



Ancillary analyses

Univariate logistic regression analysis for IR

Achievement of IR in treated patients was not influenced by age, sex, performance status, diagnosis, time from diagnosis to HSCT, donor/patient sex and NK-alloreactivity combination and disease status at HSCT.

Table 8: Univariate logistic regression analysis for immune reconstitution

Variable	Immune reconstitution		
	Odds ratio	95% CI	p value
Patient age ≥ median versus < median	1.45	0.26 to 8.01	0.66
Patient gender male versus female	0.58	0.10 to 3.32	0.54
Karnofsky performance status ≥ 90 versus < 90	2.66	0.35 to 20.49	0.33
Diagnosis AML versus other diagnoses	0.69	0.12 to 3.78	0.67
Time from diagnosis to HSCT ≥ 12 versus < 12 months	1.92	0.30 to 12.05	0.48
Donor/patient sex combination female/male versus other combinations	0.24	0.03 to 2.12	0.17
Donor/patient NK alloreactivity yes versus no	2.40	0.36 to 15.94	0.35
Disease status at HSCT remission versus relapse	1.33	0.20 to 8.49	0.76

Furthermore, there was no difference in both proportion of IR and time to IR recorded according to patient age, gender, performance status, diagnosis, time from diagnosis to transplantation, donor/patient sex combination and disease status at transplantation.

Persistence of efficacy and/or tolerance effects

Recently, MM-TK cells with preserved sensitivity to GCV have been found to persist for up to 14 years in 14 long-term survivor patients. MM-TK cells were detected also in patients who were successfully

treated with GCV for GvHD, thus confirming the GCV selectivity only for proliferating MM-TK cells (Oliveira et al, 2013).

Notably, in all analyzed patients, MM-TK cells could be retrieved from all memory T-cell subsets, which proved equally sensitive to GCV even at these long-term follow-up.

Comparison with historical control data – combined TK007 and TK008 patient population vs EBMT registry patients

Combined TK007 and TK008 patient population

As this has already been addressed for the most part earlier in this report, a brief summary is provided here. For the MM-TK group, this comprised of 30 patients from the phase I/II trial TK007 and 15 patients in the experimental arm of the ongoing phase III trial TK008 of whom clinical data were available. The TK007 trial included patients with various types of high-risk hematologic malignancies, while TK008 trial encompassed patients with acute myeloid leukaemia (AML) or acute lymphoblastic leukaemia (ALL) in first complete remission (CR1), second CR (CR2) or third CR (CR3) or with advanced or primary refractory disease (Relapse), or with secondary AML (sAML) in CR1.

The MM-TK treatment plan has also been described earlier in this report.

EBMT registry patients

Inclusion criteria for the pair-matched analysis encompassed haploidentical transplants performed from 2000 to 2013 in adult patients diagnosed with AML/ALL/sAML in complete remission or relapse at transplantation. Eligibility criteria were verified and confirmed in the EBMT Registry for 853 patients with consolidated follow-up and carefully monitored data, including 400 cases (47%) who had received a T-cell depleted HSCT without any add-back strategy and 453 (53%) a T-cell replete HSCT followed by cyclophosphamide and immunosuppression with calcineurin inhibitors and mycophenolate. The applied strategy to select the control group from the EBMT registry is agreed.

The matching strategy

To equate the distribution of baseline characteristics between the MM-TK and control group and to reduce bias in treatment effect estimation, a pair-matched analysis was performed.

This analysis, in which pairs of MM-TK and control subjects sharing similar baseline characteristics were formed, used the following parameters as pair matching factors:

• patient age (plus or minus 3 years)

• diagnosis (AML, ALL and sAML)

• disease status at HSCT (CR1, CR2, CR3 or relapse)

• time from diagnosis to HSCT (plus or minus 3 months)

The planned ratio of MM-TK patients to control patients was one to four.

The selection of these four matching factors was based on their well-recognized prognostic relevance in the transplant field for acute leukaemia, with younger patients having better prognosis than older patients, where AML cases have better outcomes than ALL and sAML cases, patients with CR1 at transplant have a better prognosis than others and patients with a short time from diagnosis have better outcomes than those with a long time from diagnosis (Cornelissen, 2012).

Outcomes and methodology

Efficacy outcome measures of this pair-matched analysis were OS, LFS, NRM and relapse incidence (RI). Cumulative incidence rates of chronic graft-versus-host disease (cGVHD) were also analysed (see

safety section for results). OS was defined as time to death from all causes. LFS was defined as time to death or first occurrence of disease relapse (or progression for patients not in CR at HSCT), whichever occurred first. In leukaemia, the term LFS is equivalent to disease (or progression)-free survival (DFS/PFS). NRM was defined as death without evidence of prior relapse (or progression).

Rates at points in time with corresponding 95% confidence intervals (CI) for LFS and OS were estimated by the product-limit method of Kaplan-Meier. Cumulative incidence functions were used to estimate rates at points in time with 95% CI for NRM, RI and cGVHD. Comparisons between curves were performed using log-rank test for survival data and Gray test for cumulative incidence curves. Competing risks were death for RI, relapse for NRM, relapse or death for cGvHD. In the first part of the analysis, baseline characteristics of the four groups (PT-Cy, TCD < 2005, TCD > 2005 and TK) were compared using the chi-square statistic test for categorical data and the Kruskal-Wallis test for continuous variables. Pairwise comparisons between the MM-TK group and control group were stratified using a mixed effects Cox model. All tests were two-sided with type I error rate fixed at 0.05. Statistical analyses were performed with IBM SPSS statistics version 22 and R 3.1.0 (R Development Core Team, Vienna, Austria) software packages.

Matched pairs

Overall, 37 TK-treated patients (23 from TK007 trial and 14 from TK008 trial) matched with 140 controls (71 from PT-Cy cohort and 69 from TCD cohort transplanted between 2005 and 2013). The choice of the Applicant to focus on the results from patients who underwent a transplantation with a TCD graft after 2005 or to whom post-transplant cyclophosphamide was applied is considered acceptable. Specifically regarding the matching, 33 TK patients were matched with four control patients, two TK patients with three control patients and one TK patient with two control patients. For three TK patients, there was no identified matching partner in the control group. See table 26 for baseline characteristics of the MM-TK treated and the control patient population.

Table 9: Baseline characteristics of the MM-TK treated and the control patient population

Pair-matched analysis		Control group (n=140)		TK group (n=37)	
Year of HSCT Median (Range)		2011 (00 - 13)		2007 (02 - 13)	
Duration of follow-up Median in months (Range)		16.9 (1.5 - 97.3)		43.2 (3.6 - 120)	
Patient age Median in years (Range)		43 (18 - 71)		43 (20 - 66)	
Time from diagnosis to HSCT Median in months (Range)		7.4 (2.5 - 54.7)		7.9 (2.2 - 52.9)	
Diagnosis	AML	102	73%	27	73%
	ALL	18	13%	5	13%
	sAML	20	14%	5	13%
Status at HSCT	CR1	64	46%	16	43%
	CR2	36	26%	10	27%
	CR3	2	1%	1	3%
	Relapse	38	27%	10	27%
Patient gender	Male	72	51%	17	46%
	Female	68	49%	20	54%
Donor gender	Male	72	52%	25	70%
	Female	67	48%	11	30%
	Missing		-	1	-
Female D → Male P	No	105	76%	32	89%
	Yes	33	24%	4	11%
	Missing	2	-	1	-
CMV D / P serostatus	D- / P-	16	12%	6	18%
	D+ / P-	11	8%	-	-
	D- / P+	15	11%	4	12%
	D+ / P+	88	68%	24	70%
	missing	10	-	3	-
Conditioning regimen	Busulfan, Cyclophosphamide +/- AraC	19	14%	-	-
	Busulfan, Fludarabine	3	2%	-	-
	Thiotepa, Busulfan, Fludarabine	22	16%	-	-
	Cyclophosphamide, Fludarabine	11	8%	1	3%
	Fludarabine, Melphalan +/- Treosulfan	18	13%	-	-
	Fludarabine, Melphalan, Thiotepa	-	-	16	43%
	Fludarabine, Treosulfan +/- Thiotepa, TBI	-	-	15	40%
	Fludarabine, Thiotepa, TBI			5	14%
	Fludarabine (o Cyclophosphamide), TBI	51	36%	-	-
	Other chemotherapy or TBI	16	11%	-	-
Source of stem cells	Bone marrow	41	29%	-	-
	Peripheral blood	94	67%	37	100%
	Both	5	4%	-	-
In vivo T-cell depletion	No	65	46%	2	5%
	Antithymocyte globulin	68	49%	35	95%
	Campath	7	5%	-	-

In the control group, there were 11 early deaths (8%) within the first 3 weeks after HSCT, due to veno-occlusive disease (n=2), infection (n=5), interstitial pneumonia (n=1), GvHD (n=1) and early relapse (n=2).

Importantly, baseline characteristics of the patients in the control group and the MM-TK-treated patients selected for the matched-pair analyses appear similar. Imbalances in baseline characteristics that were not included in the matching strategy are present (median year of transplant, gender mismatch, stem cell source, conditioning regimen and the type of in vivo T-cell depletion), which is not unexpected for this type of pair-matched cross study comparison.

Importantly, the here presented control patient population has not been matched to the MM-TK population for events occurring early after HSCT that have resulted in loss of eligibility for MM-TK administration. These events can be for instance early relapse, early death or graft failure. In study

TK007, 22 out of 52 (42.3%) enrolled and transplanted patients did not receive TK treatment due to: early death (n=12) occurring up to 55 days after HSCT, graft failure/rejection (n=7) and prolonged administration of ganciclovir or immunosuppressive therapy (n=3).

For better matching the control with the MM-TK groups for early post-transplant events, a 21-day post-transplant 'landmark' analysis was provided. In this analysis patients were not only matched for the main prognostic baseline characteristics (as in the matched-pair analysis), but were also matched, to some extent, for early post-transplant events that would have prohibited the patients who underwent a haplo-HSCT to receive MM-TK cells. Patients who either died or relapsed before day 21 were excluded from both populations. The excluded patients were 13 (11 for death and 2 for relapse) in the control group and one (for relapse) in the TK group. There were 4 cases experiencing early graft failure (lack of myeloid engraftment) in the control group, but these had already been excluded because they died before day 21. Upon request the Applicant clarified that the matching of the MM-TK patient-control was maintained, which was also evident from the provided baseline characteristics of the populations in the landmark analysis.

In an alternative analysis aiming to decrease the uncertainty regarding the adequate matching of patients for early post HSCT events, MM-TK and control patients who were alive and relapse-free were matched at the 21 day post HSCT time point. The same matching parameters were used as for the initial matched-pair analysis. In this analysis 139 control patients (70 T-cell replete and 69 T-cell depleted grafts) were matched with 36 TK-treated patients. In addition, in order to minimize bias due to differences in the timing of MM-TK infusion, 3 further 'sensitivity' were performed in by excluding patients who had died or relapsed before 4, 6 and 8 weeks after transplant.

Results

Matched-pair analysis at time of HSCT

Overall survival (OS) at 1-year was significantly improved in the MM-TK-group compared with the control group (p=0.01). The survival rates were 49% and 37% for MM-TK- and control group, respectively. The NRM at 1-year was also improved upon treatment with MM-TK, with 43% for the control group and 22% for the MM-TK-group (p=0.014). A difference in favour of the TK-group could also be observed for the 1-year incidence of cGvHD with 25% for the control group vs 6% for the TK-group (p=0.04). The LFS and the RI were not different between the groups (see also Table 27), because NRM and relapse are competing risk events and relapse events occur later than NRM events. Together the data suggest that the benefit seen in OS is mainly driven by a reduction in the NRM.

A further analysis of the NRM data in the matched pair comparison revealed that in the control group 34 of 140 (24%) patients died due to infection and 8 of 140 (6%) succumbed due to GvHD. In the MM-TK population, 4 (11%) patients died because of infection and no patient died due to GvHD. This suggests that the reduction in NRM mortality in the MM-TK population is caused both by a reduction in death due to infection and due to GvHD.

To explore the impact of MM-TK cells given after relapse (DLI setting, was allowed in TK007, but not in TK008) on OS, the 8 patients who subsequently received MM-TK as DLI, were excluded from the MM-TK group of the pair-matched analysis. The remaining 29 MM-TK patients showed a significantly improved OS compared with the 140 control patients (stratified HR=0.46; 95% CI, 0.23 to 0.93; p=0.03; 1-year, 49% vs. 37%), with no further deaths observed in the TK group after 1 year (with a three-year median follow-up time). In this analysis the matching between the groups was not maintained as the matched-pair counter parts of the excluded MM-TK patients were not removed from the control population. Despite this omission, it indeed appears that influence of the MM-TK administered as DLI to some patients on the survival curve/results is limited.

'Landmark' analysis

The data from this analysis show that the MM-TK treated patients benefited in terms of 1-year OS (40% vs 51% (p=0.03)) and 1-year NRM (42% vs 23% (p=0.04)), for control vs MM-TK treated patients, respectively. Also the difference in favour of the MM-TK-group for the 1-year incidence of cGvHD could be observed (28% vs 7% p=0.04) (Table 28). Again no difference was observed in LFS and the chance for relapse seemed higher in the MM-TK-treated patients as compared to the control patients (p>0.05). These data are similar as in the initial matched pair analyses.

The used cut-off of 21 days for early post-transplant events prohibiting the administration of MM-TK cells was chosen based on the TK007 trial where patients were to receive the first MM-TK administration at the moment of myeloid engraftment. This was anticipated to occur before 21 days following HSCT. However, for MM-TK patients the time frame between HSCT and first MM-TK infusion varied depending on the timing of myeloid engraftment and/or need for ganciclovir therapy to treat active CMV infection. In the TK007 trial, MM-TK treated patients received their first infusion of MM-TK cells at a median of 43 days from the date of HSCT ranging from 16 days to 75 days post-HSCT (such data are not provided for the TK008 patients). In an attempt to address this uncertainty, the Applicant has provided an additional analysis by adjusting the calculation of the survival time. For the control patients, the calculation of survival time started at day 21 post HSCT (i.e. similar to the landmark analysis), thus assuming that these patients would have received MM-TK at this time point (if they had been in an MM-TK trial). For the MM-TK patients the calculation started at the actual day of first MM-TK infusion. In this analysis an improvement was seen in OS (stratified p=0.045; 1-year rate, 47% vs. 40%) and in NRM (p=0.012; 1-year rate, 26% vs. 42%) for the TK group compared with the control group. However, in a further Cox regression analysis that included the MM-TK infusion as a time-dependent covariate, the 95% CI of the hazard ratio for OS (0.59; 95% CI, 0.32-1.09; stratified p=0.09) and NRM (0.48; 95% CI, 0.19-1.17; p=0.11) contain 1. This analysis can be seen as a form of sensitivity analysis on the landmark approach. However, the start of calculation of survival time (relative to HSCT) is different between the treatment arms, and it is thus not clear if this is a conservative approach or not.

Matched–pair at 21 days post HSCT

In order to address the uncertainty regarding the impact of early post-transplant events, patients were control and MM-TK patients were matched at day 21 post HSCT. This analysis showed an absolute increase of 17% in 1-year OS rate for the MM-TK group compared with the control group (51% vs 34%; p=0.007) and a benefit in 1-year NRM (20% vs 46%; p=0.003) and chronic GvHD incidence (6% vs 23%; p=0.02) for MM-TK treated patients (Table 29). As noted before, no benefit is observed in LFS and the chance for relapse seemed higher in the MM-TK-treated patients as compared to the control patients, but it should be kept in mind that relapse and NRM are competing risks.

The applicant performed a matched-pair analyses with MM-TK treated patients. Of the 45 MM-TK treated subjects, 37 patients (23 from TK007 trial and 14 from TK008 trial) were matched to 140 controls (71 from PT-Cy cohort and 69 from TCD cohort transplanted between 2005 and 2013). Efficacy outcome measures of this pair-matched analysis were overall survival (OS), leukaemia-free survival (LFS), non-relapse mortality (NRM) and relapse incidence (RI). Cumulative incidence rates of chronic graft-versus-host disease (cGVHD) were also analysed. The details of this comparison, such as the strategy for the matched-pair analyses, the patient characteristics and outcomes will be discussed at the end of the discussion of clinical efficacy section.

Table 10: Summary of the overall outcomes at 1-year of the pair-matched analysis between TK-treated patients and EBMT control patients

Pair-matched analysis	Relapse incidence (RI)	Non-relapse mortality (NRM)	Leukemia-free survival (LFS)	Overall survival (OS)	Chronic GvHD
Controls (n=140) (95% CI)	**22%** (15-31)	**43%** (34-52)	**35%** (25-44)	**37%** (28-46)	**25%** (17-33)
TK (n=37) (95% CI)	**41%** (23-57)	**22%** (14-31)	**37%** (20-54)	**49%** (32-67)	**6%** (1-19)
p-value (stratified)	*0.30*	*0.014*	*0.19*	*0.01*	*0.04*
Hazard ratio (HR)	*1.47*	*0.34*	*0.71*	*0.48*	*0.15*
(95% CI)	*(0.71-3.04)*	*(0.14-0.81)*	*(0.42-1.19)*	*(0.26-0.86)*	*(0.05- 0.91)*

P<0.05 was considered significant.

Table 11: Summary of the overall outcomes at 1–year based on a conditional landmark analysis performed 21 days after HSCT

Pair-matched analysis Landmark analysis at 21 days after HSCT	Relapse incidence (RI)	Non-relapse mortality (NRM)	Leukemia-free survival (LFS)	Overall survival (OS)
Controls (n=127) (95% CI)	**21%** (14-30)	**42%** (32-52)	**37%** (27-47)	**40%** (30-50)
TK (n=36) (95% CI)	**39%** (22-56)	**23%** (15-33)	**38%** (21-55)	**51%** (33-69)
p-value (stratified)	*0.28*	*0.04*	*0.35*	*0.03*

This analysis reported in table 28 excluded patients who died or relapsed before day 21, as was an eligibility criterion for treatment with MM-TK cells (next to the absence of e.g. graft failure or early relapse). Thirteen patients in the control group and one patient in the TK group were excluded for this landmark analyses.

Upon request the Applicant provided an additional pair-matched analysis where patients were matched at week 3 after HSCT. This was done to better match the patients populations for early (within 3 weeks) post-transplant events that could have prohibited the administration of MM-TK (such as death, relapse, non-myeloid engraftment, CMV infection requiring GCV treatment). At this 3 week time point patients had to be alive and relapse free. The results of this new comparison are shown in table 29.

Table 12: Summary of the overall outcomes at 1–year based on a matched-pair analysis performed 21 days after HSCT

New pair-matched analysis 1-year outcomes Alive and relapse free at 21 days	Relapse incidence (RI)*	Leukemia-free survival (LFS)	Non-relapse mortality (NRM)	Overall survival (OS)	Chronic GvHD
Controls (n=139) (95% CI)	21% (14-29)	33% (24-43)	46% (36-55)	34% (25-44)	23% (15-32)
TK (n=36) (95% CI)	42% (22-56)	38% (21-56)	20% (8-36)	51% (33-69)	6% (1-19)
*p-value^*	*0.06*	*0.12*	*0.003*	*0.007*	*0.02*

**RI and NRM are competing risk events (i.e., when one competing event occurs, patients are no longer at risk for the other event, with those with shorter survival being less likely to develop relapse) and NRM events tend to occur earlier than relapse events. ^Cox test p-value stratified on match group for LFS and OS and Gray test p-value for RI, NRM and chronic GvHD*

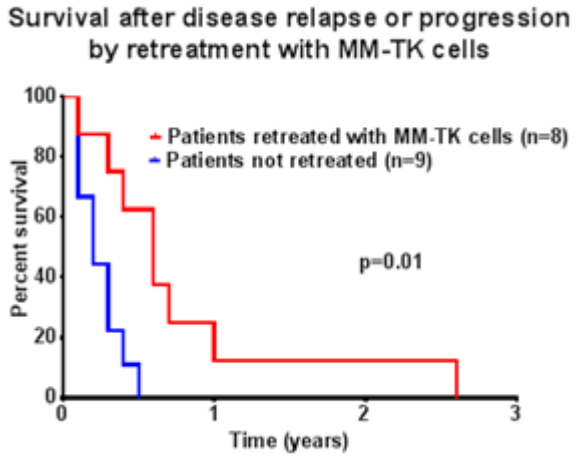
The new pair-matched analysis showed an absolute increase of 17% in one-year OS rate for the TK group compared with the control group (51% vs 34%; p=0.007), which is larger than seen in previous 'landmark' analysis (51% vs 40%, p=0.03).

To address the uncertainty regarding the appropriateness of the 3 weeks as cut off for early post-transplant events, the Applicant was requested using 4, 6 and 8 weeks post HSCT as cut off. For these analysis, patients who had died or relapsed before these time points were excluded. These analyses revealed similar significant improvements in OS, NRM and chronic GvHD in favour of TK-treated subjects as compared to control patients as seen with the analysis using the 3 week cut off value. Also the difference in relapse incidence and leukaemia free survival did not appear to change substantially, because NRM and relapse are competing risk events and relapse events occur later than NRM events.

Use of the MM-TK cells as DLI

As rescue treatment for disease relapse or progression, eight patients were rechallenged with an additional 16 infusions of MM-TK cells, including six patients who had subsequent HSCT, four unmanipulated lymphocytes and four chemotherapies. In contrast, 9 patients with disease relapse/progression were not retreated with MM-TK cells, but received subsequent T-replete HSCT (n=2), unmanipulated lymphocytes (n=4) and/or chemotherapy (n=4). All patients whose disease relapsed or progressed died after a median post-relapse/progression survival time (computed from date of relapse or progression to death) of 123 days (95% CI, 55 to 209). Compared to nine patients who were not retreated, eight patients who were rechallenged with MM-TK cells had significantly improved post-relapse/progression survival rates (HR=0.19; 95% CI, 0.05 to 0.72; p=0.01) Figure 19).

Figure 19: Survival time after disease relapse or progression according to retreatment with MM-TK cells



Although the results are suggestive for a favourable effect of MM-TK treatment as DLI upon relapse or progressive disease, no fair comparison between the 8 retreated and the 9 not retreated can be made, as this is not a comparison between randomized groups. Therefore, selection bias could be present.

Moreover, the objective of DLI is to re-induce remission in patients who have a relapse post-transplant. This is out of the scope of the current application meaning that these data should be considered as supportive.

Summary of main study(ies)

The following table summarises the efficacy results from the TK007 study in which most of the MM-TK treated patients were treated (n=30 out of 45 MM-TK treated patients with clinical data available). This summary should be read in conjunction with the discussion on clinical efficacy as well as the benefit/risk assessment (see later sections).

Table 13: Summary of efficacy for trial TK007

Title: A phase I-II study: infusion of donor lymphocytes transduced with the suicide gene HSV-TK, after transplantation of allogeneic T-depleted stem cells from a haploidentical donor in patients with hematological malignancies.		
Study identifier	TK007	
Design	Open-label, non-randomized, multicenter, international phase I/II study	
	Duration of main phase: Duration of enrolment:	11 years and 3 months 70 months
Hypothesis	Investigate the clinical activity in terms of IR, control of GvHD after ganciclovir use and evaluation of GvL effect of an add back strategy with the MM-TK cells given after T-cell depleted HSCT (positively selected CD34+ cells plus a fixed dose of CD3+ cells of $1x10^4$/kg).	

Treatments groups	MM-TK		The treatment phase lasted approximately four months starting 21 to 49 days after HSCT. The initial dose of MM-TK cells was given in absence of IR and/or GvHD. The treatment plan consisted of up to 4 monthly IV infusions given at the following doses: - first dose: $1x10^6$ or $1x10^7$ cells/kg - second dose: $1x10^7$ cells/kg - third dose: $1x10^6$ cells/kg plus interleukin-2 ($1x10^6$ IU/m2 subcutaneously for five days) - fourth dose: $1x10^7$ cells/kg plus interleukin-2 ($1x10^6$ IU/m2 subcutaneously for five days). Long-term follow-up was related to patient outcome.
Endpoints and definitions	Immune reconstitution (Prim.)	IR	Number (%) of patients reaching CD3+ ≥ 100/µL (and/or CD4+ and/or CD8+ cell count ≥ 50/µL).
	Disease Free Survival/Progression Free Survival	DFS/PFS	Percentage of patients without disease relapse/progression since HSCT at 1-5 years.
	Overall survival (Sec.)	OS	Percentage of patients alive (at 1-5-10 years).
	Non-relapse mortality (Sec.)	NRM	Number (%) of patients died without previous disease relapse-progression (at 1 year and at 5 years).
	Infectious events (Sec.)	-	Median number of infectious adverse events per patients in ITT within observation time (median 3.8 months).
Data cut-off	December 2013		

Results and Analysis

Analysis description	**Primary Analysis**	
Analysis population and time point description	Enrolled patients / Intent tot treat (ITT)= all patients receiving HSCT **/** Treated population = number of patients treated with MM-TK cells.**	
Descriptive statistics and estimate variability	Treatment group	MM-TK
	Number of subjects	(n=57) **/** n=52 **/** n=30**
	IR	23/52 (44%) **/** 23/30 (77%)
	DFS/PFS	21%$ **/** 30%$ 17%* **/** 27%*
	OS	17% (ITT)***
	NRM	26/52 (50%)$ * **/** 9/30 (30%)$ *
	Infectious events	Median # infections: 5 (range 1-20; median duration per event: 11 days (range 1-84) (ITT) Median # of viral infections: 3 (range 1-7; median duration per event is 15 days (range 1-84) (ITT)

*$ at 1 year * at 5 years; *** at 10 years; HSV-K, Herpes Simplex Thymidine Kinase; IR, immune reconstitution; GvHD, graft versus host disease; GvL, graft versus leukaemia; HSCT, haematopoietic stem cell transplantations; NRM, non-relapse mortality; OS, overall survival; ITT, intention to treat; DLI, donor lymphocyte infusion.*

Analysis performed across trials (pooled analyses and meta-analysis)

Table 31 lists the main baseline patient and disease characteristics for the 52 patients enrolled in the TK007 study and of the 17 patients randomized in the experimental arm A of the phase III TK008 study.

Table 14: Patient and disease characteristics in patients enrolled in the phase I/II TK007 trial and those enrolled in the experimental arm of the phase III TK008 trial

Variable	TK007 n=52 (%)	TK008 n=17 (%)
Patient age		
Median in years	49	37
Range	17 - 66	19 - 65
25th - 75th percentile	35 - 57	29 - 53
≥ 60 years	12 (23)	3 (18)
Gender		
Male	22 (42)	11 (65)
Female	30 (58)	6 (35)
Karnofsky performance status		
100	37 (71)	16 (94)
90	3 (6)	-
80	8 (15)	1 (6)
70	3 (6)	-
Not available	1 (2)	-
Time from diagnosis to HSCT		
Median in months	10.6	[illegible]
≥ 12 months	22 (42)	6 (35)
< 12 months	30 (58)	10 (59)
Not available	-	1 (6)
Diagnosis		
AML	26 (50)	13 (76)
Secondary AML	10 (19)	-
MDS/RAEB/RAEB-T	7 (13)	-
HD/NHL	1 (2)	-
ALL	3 (6)	4 (24)
CML	1 (2)	-
Biphenotipic leukemia	1 (2)	-
Complete remission at HSCT	31 (60)	15 (88)
First	16	10
Second	11	5
Third	4	-
Relapsed/progressive disease at HSCT	21 (40)	2 (12)

The median time to first infusion in TK008 was 28 days (95% CI, 25 to 30) after HSCT and the median interval time between the first and the subsequent cell doses administered was 32 days (95% CI, 30 to 40). In all, 11 of 15 treated patients (73%) achieved IR. This % of patients achieving IR is similar as observed in TK007, i.e. 77%. The median time to attain IR in TK008 was 26 days (95% CI, 14 to 34) after last infusion of MM-TK cells, which is similar as observed in TK007.

Clinical studies in special populations

	Age 65-74 (Older subjects number /total number)	Age 75-84 (Older subjects number /total number)	Age 85+ (Older subjects number /total number)
Controlled Trials			
Non Controlled Trials	**1**		

Supportive study(ies)

In addition to TK007, a phase III study, TK008, is currently ongoing (start in February 2010; 170 patients planned; as per cut-off date of December 2013 a total of 27 patients were enrolled, in March 2016 a total of 50 have been randomly assigned). The primary objective of TK008 is to compare the efficacy in terms of disease/progression-free survival of an add back strategy with the use of MM-TK cells after T-cell depleted HSCT (consisting of positively selected CD34+ cells plus a fixed dose of CD3+ cells of $1x10^4$/kg) versus either T-cell depleted HSCT or T-cell replete (unmanipulated) HSCT followed by high-dose cyclophosphamide.

PHASE III TK008 STUDY: PRELIMINARY RESULTS

Preliminary efficacy and safety data are provided for the first 17 patients consecutively randomized in the experimental arm A.

During treatment phase, a total of 31 infusions of MM-TK cells were given to 15 patients, with five patients (33%) having one infusion, five patients (33%) two infusions, four patients (27%) three infusions and one (7%) four infusions, which was the maximum number of infusions planned by study protocol. The median number of MM-TK cell doses per patient was two (95% CI, 1 to 3; range, 1 to 4), while the median cumulative cell dose was $2.4x10^7$/kg (range, 1 to 3.9).

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The application for Zalmoxis was initially based on the single arm, phase I/II study TK007 with the primary objective of immune reconstitution (IR) defined as CD3+ cells ≥100 per µL by MM-TK treatment. The randomised controlled phase III study TK008 with disease free survival (DFS)/progression free survival (PFS) as primary endpoint, is ongoing. The overall purpose of MM-TK treatment is IR leading to a lower NRM and a lower relapse incidence (RI), resulting in improved OS. The study population concern adult patients with haematological malignancies at high risk of relapse who had received a haematopoietic stem cell transplantation (HSCT) from a HLA mismatched (haploidentical) donor for 2 or 3 loci and who were in good clinical condition at the time of MM-TK administration. In TK008 the patient population was restricted to subjects with acute myeloid leukaemia (AML), secondary AML or acute lymphoblastic leukaemia (ALL).

The aim of study TK007, which is a phase I/II, single arm study in patients with high risk haematological malignancies was to assess the clinical activity in terms of IR, control of GvHD after GCV use and evaluation of GvL effect of an add back strategy with genetically manipulated T-cells after T-cell depleted haploidentical HSCT. The genetically manipulated T-cells, or MM-TK cells, are retrovirally transduced to express HSV-TK, a suicide gene, which would enable termination of the graft versus-host-disease that may arise upon administration of MM-TK cells. In total 57 patients were enrolled in TK007 of whom 52 patients transplanted. Eventually, 30 patients were treated with MM-TK cells post-HSCT in TK007.

The Applicant has not performed dose response studies specifically, although TK007 contained a dose-response component with two doses transplanted, i.e. $1x10^6$ cells/kg or $1x10^7$ cells/kg IV. The Applicant did perform exposure-response analysis.

The clinical benefit of MM-TK cells after haploidentical HSCT was to be further established by comparing patient-level data from the TK007 and TK008 trials with patient-level data of historical controls of the EBMT registry.

Based on the defined inclusion and exclusion criteria, the studied patient population concerns adult patients with haematological malignancies at high risk of relapse who had received a HSCT from a HLA mismatched (haploidentical) donor for 2 or 3 loci and who were in good clinical condition at the time of MM-TK administration. The criteria for the designation of a “high-risk” haematological patient may vary in time, in particular considering evolving treatment modalities and scientific considerations. However, only patients with high risk haematological disease are to be selected as a candidate for haploidentical HSCT, therefore, no specific criteria to define the “high-risk” patient are required.

The following doses were applied in TK007: 1st dose: $1x10^6$ or $1x10^7$ cells/kg; 2nd dose: $1x10^7$ cells/kg; 3rd dose: $1x10^6$ cells/kg plus interleukin-2 (IL-2) (1×10^6 IU/m^2 subcutaneously for 5 days); 4th dose: $1x10^7$ cells/kg plus IL-2 (1×10^6 IU/m^2 subcutaneously for 5 days). The recombinant interleukin-2 at dosage of $1x10^6$ IU/m^2 subcutaneously administered for five days was planned in the TK007 study in combination with the third and the fourth dose of MM-TK cells infusions to increase the differentiation and proliferation of T-cells and anti-tumour effects. Notably, the impact of adding IL-2 after the first two infusions of MM-TK cells was deemed negligible and, therefore, IL-2 was not included in the treatment plan of the phase III trial TK008.

In TK008 the same schedule was used, though the planned cell dose was $1x10^7$ cells/kg, without the use of IL-2.

The endpoints fit the objectives and the overall aim of the studies. In absence of a standard cut-off for IR after T-cell depleted transplantation, IR was empirically defined in TK007 as a CD3+ cell count ≥100/µL (and/or CD4+ and/or CD8+ ≥50/µL). According to NCI-CTC AEs criteria, these values roughly correspond to the upper limit that defines the grade 4 lymphocytopenia (below 200/µL for lymphocyte count decreased or below 50/µL for CD4 count decreased) and delimits the initial period of life-threatening immunodeficiency immediately after T-cell depleted HSCT.

The optimal dose of MM-TK cells cannot be clearly established based on the data in study TK007, but even so the recommended dose in TK008 is $1x10^7$ cells/kg of MM-TK. According to the Applicant, the selection of $1.0x10^7$/kg to be used in the phase III study was also based on the relationship between the anti-leukaemia effect and the cumulative dose of MM-TK cells administered in TK007.

In total 57 patients have been enrolled in the TK007 study, which is a much larger number that the 18 subjects needed for the Simon design. This can be explained by the fact that only patients who had received at least one infusion of MM-TK cells were evaluable for the primary endpoint. Thus of 57 patients included in TK007, 30 patients were evaluable for IR with 7 evaluable patients in stage 1, 11 evaluable patients in stage 2 as required by the Simon design and 12 evaluable patients as required in the equivalence part of the study. Therefore, no surplus of patients were analysed for the primary outcome and the increased recruitment is unlikely to refer to opportunistically increasing the size of the trial to obtain statistical significance.

Statistical methods used for continuous, categorical and time-to-event outcomes are considered standard and acceptable. Secondary HSCT was not considered an event, but given the aim of the study, this is considered acceptable.

Considering the absence of an internal control in the study, a comparison with historical controls was needed. To this end, the Applicant formally approached the EBMT society to request the use of their patient database for composing an appropriate control group.

Several protocol deviations have been noted. From the information provided it is understood that these protocol deviations were mainly caused by decisions of the treating physicians and in one case by the use of a non-protocol laboratory method to determine the number of CD3+ cells. It is difficult to ascertain the impact of these deviations on the study conduct and results, though considering the explanations provided, it is assumed to be limited.

There were no significant differences in baseline risk factors between patients who received MM-TK cells and patients who did not, except for the number of patients with progressive/relapsed disease at HSCT. The patient subgroups, including the number of the patients involved per subgroup, can be agreed. However, the subgroups consist of a (very) limited number of patients and the various subgroups are not all necessarily relevant to establish the efficacy and safety of the MM-TK as adjunctive treatment in haploidentical HSCT of adult patients with high-risk haematological malignancies.

Efficacy data and additional analyses

Overall, 23 of 30 MM-TK treated patients (77%; 95% CI, 59 to 88) reached the protocol-defined IR. This percentage of IR seems to indicate the feasibility of the treatment in particular considering the fact that a median of 1 infusion was required to establish IR with a median cumulative dose of $1x10^7$ MM-TK cells/kg. It is, furthermore, reassuring that baseline characteristics were equally distributed between MM-TK treated patients that reached IR and those that did not. Also there was no difference in starting dose ($1x10^6$/kg or $1x10^7$ cells/kg), cumulative cell dose (median, $1.3x10^7$/kg versus $1.0x10^7$ cells/kg), number of infusions (median, one for both) or fresh vs frozen MM-TK cells between the IR (n=23) vs the non-IR patient group (n=7), respectively.

According to the Applicant, only three patients (TK3-11-52) received the third and the fourth MM-TK infusion and only one patient (TK11) received IL-2, which was associated with toxicity. None of the three patients who received the third and the fourth MM-TK dose reached immune reconstitution. Two additional patients received two non-protocol-mandated IL-2 courses. The impact of adding IL-2 after the first two infusions of MM-TK cells was deemed negligible and, therefore, IL-2 was not included in the treatment plan of the phase III trial TK008.

GvHD is an expected complication/adverse event following allogeneic HSCT and/or infusion of allogeneic T-cells. To enable termination of the GvHD that may arise upon administration of T-cells, the MM-TK cells were genetically manipulated by retroviral transduction to express the Herpes Simplex Thymidine Kinase (HSV-TK) suicide gene. This could be considered as the main possible additional benefit of MM-TK in comparison to non-genetically modified T-cell grafts that are used as an adjunctive treatment upon relapse in the post-allogeneic transplant setting in high risk patients. Indeed, the GvHD that occurred upon MM-TK administration all resolved after activation of the suicide gene upon administration of ganciclovir to the patients.

The adjunctive treatment effect of the MM-TK cells given after T-cell depleted haploidentical HSCT was determined by relating the MM-TK results with historical EBMT control data. These were derived from patients undergoing haploidentical transplantations performed according to the two most commonly used modalities of GvHD prevention: T-cell depleted transplant without any add-back strategy (TCD cohort) and T-cell replete (unmanipulated) transplant followed by post-graft infusion of cyclophosphamide and immune suppression with a calcineurin inhibitor and mycophenolate (PT-Cy cohort). Both options are also included in the control arm of the ongoing phase III trial TK008.

In total 47 patients have been treated with MM-TK cells (n=30 in TK007 and n=17 in TK008) of whom of 45 patients clinical data were available (n=30 in TK007 and n=15 in TK008). Of these subjects, 37 MM-TK-treated patients (23 from TK007 trial and 14 from TK008 trial) were matched with 140 controls from the EBMT database (71 from PT-Cy cohort and 69 from TCD cohort transplanted between 2005 and 2013). The 4 matching parameters used, i.e. age, diagnosis, disease status at transplant and interval between diagnosis and transplant, can be considered appropriate as these are the major prognostic parameters for survival.

The results from this matched-pair analyses showed that the MM-TK-treated patients seem to benefit in terms of 1 year OS (i.e. 49% for MM-TK vs 37% for control, p=0.01) and 1-year NRM (i.e. 22% for

MM-TK vs 43% for control, p=0.014), but that there is no advantage in 1-year LFS (i.e. 37% for MM-TK vs 35% for control) and the chance for relapse (i.e. 41% for MM-TK vs 22% for control).

However, and more importantly, in this matched-pair analyses patients are not matched for (early) post-HSCT events such as early death, relapse of non-myeloid engraftment. Therefore a landmark analysis was performed on the matched pair population. Patients who either died, relapsed or experiencing early graft failure (lack of myeloid engraftment) before day 21 were excluded from the matched-pair populations. This resulted in a population of 36 MM-TK patients and 127 control patients. The matching of the major prognostic baseline parameters between MM-TK and control patients was maintained upon this additional selection step.

The data from this landmark analysis show that the MM-TK treated patients that had survived the first 3 weeks post-transplant benefited in terms of 1 year OS (40% vs 51% (p=0.03)) and 1-year NRM (42% vs 23% (p=0.04)), for control vs MM-TK treated patients, respectively. Also here there was no significant difference regarding LFS and the chance for relapse.

In an alternative analysis aiming to decrease the uncertainty regarding the adequate matching of patients for early post HSCT events, MM-TK and control patients who were alive and relapse-free were matched at the 21 day post HSCT time point. The same matching parameters were used as for the initial matched-pair analysis. In this analysis 139 control patients (70 T-cell replete and 69 T-cell depleted grafts) were matched with 36 TK-treated patients. The data from this analysis showed an absolute increase of 17% in 1-year OS rate for the MM-TK group compared with the control group (51% vs 34%; p=0.007) and a benefit in 1-year NRM (20% vs 46%; p=0.003) for MM-TK treated patients. Again, also here no significant difference regarding LFS was observed while chance for relapse seemed higher in the MM-TK-treated patients as compared to the control patients. Notably, relapse and NRM are competing risks.

In order to minimize bias due to differences in the timing of MM-TK infusion, 3 further 'sensitivity' were performed in which were excluded patients who had died or relapsed before 4, 6 and 8 weeks after transplant. These analyses revealed similar significant improvements in OS, NRM and chronic GvHD in favour of MM-TK-treated subjects as compared to control patients as seen with the analysis using the 3 week time point. Also the difference in relapse incidence and leukaemia free survival did not appear to change substantially.

The role of MM-TK cells to a (hastened) IR cannot formally be established. However, considering the temporal association between the administration of MM-TK and occurrence of IR, it is likely that MM-TK has contributed. Even though the results are suggestive of IR being an early surrogate marker for efficacy in the small uncontrolled TK007 study in terms of OS, infections and reduced NRM, this is not based on a comparison between randomised groups and thus firm conclusions cannot be drawn.

The effect of IR on relapse cannot be adequately analysed, because, as brought forward by the Applicant, NRM and relapse are competing risk events, and patients without IR tended to die before relapse could occur. However, the currently available data do not support any claim that MM-TK administration provides protection against relapse of the underlying haematological disease. This is contrast to the anti-leukemic effect reported for DLI administered upon relapse in the post-allogeneic transplant setting.

Data from an adequately controlled trial are lacking, and all data on the efficacy of MM-TK treatment comes from a cross study comparison using a historical control group. Thus all efficacy data are associated with the uncertainties inherent to cross study comparisons. The main uncertainties associated with the current comparison pertain to the low number of patients, the unknown impact of potential differences in baseline characteristics not included in the matching strategy (e.g. median year of transplant, stem cell source, conditioning regimen) and the inherent uncertainty with cross study

comparison. Albeit that it should be acknowledged that the Applicant has done their best to reduce the uncertainties associated with this comparison, and that the use of the EBMT database for obtaining control patients and the matching strategy severely reduced the uncertainties of the requested cross study comparison, and that all the pair-matched, landmark and sensitivity analyses performed, while aiming to reduce uncertainties, did not change the observed differences in OS and NRM.

In addition, the mechanism behind the reduction in NRM has not been fully explained, though it appears to be caused by a reduction in death due to infection and to GvHD. Also the long-term efficacy remains uncertain as data are very limited. However this may be accepted as NRM is the main driver of the effect of MM-TK on OS, and NRM generally occurs within the first year post transplant.

Furthermore, it is noted that the current results do not fully support the proposed aim of the MM-TK treatment, i.e. there is a positive effect on NRM reduction, but the RI is not lowered, because NRM and relapse are competing risk events and relapse events occur later than NRM events. However, the data indicate that despite the lack of a clear anti-leukemic effect, the reduction in NRM on its own is able to result in an improved survival.

Although the supportive data from TK007 on MM-TK treatment as DLI upon relapse or progressive disease are suggestive for a favourable effect, no true comparison between the 8 retreated and the 9 not retreated can be made, as this is not a comparison between randomized groups. Therefore, selection bias could be present.

Additional efficacy data needed in the context of a conditional MA

As comparative, randomised data were considered necessary the ongoing TK008 trial will allow provision of comprehensive data in order to confirm the efficacy and safety of Zalmoxis as an adjunctive treatment in haploidentical haematopoietic stem-cell transplantation of adult patients with high-risk haematological malignancies. This is a randomized phase III trial of haploidentical HCT with or without an add back strategy of HSV-Tk donor lymphocytes in patients with high risk acute leukaemia.

The primary study objective of TK008 trial is disease-free survival (DFS), defined as the time from randomization to relapse or death from any cause. This endpoint will allow to capture the protective effects of Zalmoxis treatment on clinical outcome, including non-relapse mortality (NRM) that is largely driven by infection-related and GvHD-related mortality, as well as relapse. The sample size of TK008 trial (n=170 patients, 127 in experimental arm and 43 in control arm) was calculated based on the primary study objective, which aims to demonstrate the superiority of Zalmoxis treatment after haploidentical transplantation versus the two most used haploidentical transplant platforms (T-cell depleted or T-cell replete).

It is noted that, given the findings of the pair-matched analyses, it may be questioned whether DFS best captures the benefit of the treatment. However, a change in the protocol (e.g. by changing the primary endpoint from DFS to OS) was thought not to facilitate a holistic assessment of data. Importantly, as OS is considered to be an important outcome measure. It is reassuring that, based on the clinical and statistical assumptions, the sample size provided by the Applicant, could be sufficient to also capture a statistical significant difference in this endpoint.

Furthermore a non-interventional safety and efficacy study TK011 will investigate effectiveness in real clinical practice by collecting data about the disease status and outcome of all patients treated with Zalmoxis using the EBMT registry.

2.5.4. Conclusions on the clinical efficacy

The clinical benefit of MM-TK treatment is demonstrated through extensive analyses from TK007 and preliminary data from TK008 studies. The current data indicate that the benefit of treatment in terms of OS with Zalmoxis encompasses a reduction in NRM not of anti-leukemic effect.

The CAT considers the following measures necessary to further confirm the benefit-risk of Zalmoxis in the context of a conditional MA:

In order to confirm the efficacy and safety of Zalmoxis as an adjunctive treatment in haploidentical haematopoietic stem-cell transplantation of adult patients with high-risk haematological malignancies, the MAH should submit the results of study TK008, a randomized phase III trial of haploidentical HCT with or without an add back strategy of HSV-TK donor lymphocytes in patients with high risk acute leukaemia. A final clinical study report will be submitted March 2021, within 12 months of the end of data collection. Six monthly-updates (with particular regards to the patient recruitment status) will be submitted within the PSUR.

Regarding the feasibility of TK008, it appears that the plan of the Applicant to include more centres has succeeded as 25 new centres have already confirmed their participation in TK008. This brings the number of centres to 40. Considering the increase in the number of participating centers, the completion of the study within a reasonable time frame and the submission of final study report by March 2021 seem feasible. This provides sufficient reassurance on the feasibility to provide confirmatory data, i.e. comparative results from the TK008 phase III trial. Importantly, based on the calculation provided by the Applicant, it seems that the sample size seems sufficient to also capture a statistical significant difference in OS.

Furthermore, the CAT considers the following measures necessary:

A non-Interventional PASS (study TK011) is imposed as a condition to the marketing authorisation in order to investigate the safety and the effectiveness of Zalmoxis in real clinical practice as well as long-term safety and efficacy. In the context of this study, the MAH should collect data about the disease status and outcome of all patients treated with Zalmoxis using the EBMT registry.

An updated protocol of the TK011 will be submitted for review as early as possible but no later than 6 months after the granting of the MA. Yearly reports on the data from the TK011 should be submitted within the annual renewal of the conditional marketing authorisation.

The CHMP endorse the CAT conclusion on clinical efficacy as described above.

2.6. Clinical safety

The safety assessment MM-TK cells was mainly based on clinical and laboratory evaluations from the phase I/II TK007 study (only 30 MM-TK-treated patients). The safety database is considered very limited.

In TK007 the collection of safety data was study-phase specific with only infectious adverse events (AEs) collected from the screening phase until the first infusion of MM-TK cells in order to define the safety during the peri-HSCT phase. All AEs regardless their relationship with MM-TK cells were collected during the treatment phase (which includes both the infusions of up to four monthly infusions starting from 21 to 49 days after HSCT and the 6 months of the follow up phases). Only AEs possibly related to MM-TK cells were afterwards collected. The AEs were graded according to National Cancer Institute Common Toxicity Criteria version 3.0 (NCI-CTC v. 3.0).

After each infusion of MM-TK cells, patients were monitored for IR and occurrence of GvHD. After achieving IR, patients were monitored monthly for five months for immunological parameters

(including immunophenotyping, gene-marking and functional studies), for GvHD, laboratory parameters, and safety aspects, including replication-competent retroviruses (RCR).

The monitoring for GvHD was performed through physical and clinical examinations (e.g. skin rash, intensity and number of stools of diarrhoea) and laboratory parameters (e.g. bilirubin and hepatic enzyme levels). Acute and chronic GvHD were graded according to published guidelines (Przepiorka et al, 1995; Glucksberg et al, 1974; Filipovich et al, 2005). The approach as used by the Applicant to monitor for GvHD is acceptable. It is noted that diagnosing GvHD based on the documented endpoints alone may be not straightforward, and other possible causes for isolated abnormalities should be considered and excluded, prior to diagnosis of GvHD. When in doubt, histologic examination is generally recommended. It is also noted that the Glucksberg et al. (1974) and Przepiorka et al. (1995), describe different grading systems for GvHD. However, the clinical impact of these differences is considered limited, as it is assumed that patients with (suspect) GvHD are treated by experienced physicians.

According to the Applicant, the RCR search was carried out on genomic DNA from $5x10^6$ patient's peripheral blood lymphocytes, using molecular tests (Q-PCR env), in compliance with EMEA guideline (Guideline on follow-up of patients administered with gene therapy medicinal products - EMEA/CHMP/GTWP/60436/2007), following this time schedule: at baseline (i.e. immediately before 1st MM-TK infusion), at 3 months, at 6 months, at 1 year after 1st MM-TK infusion and yearly for at least 5 years. It was planned that if the samples collected during the first year were always negative, the subsequent yearly samples were taken, but not analysed; in case of one or more positive samples, the culture test was performed for confirmation.

Patient exposure

In TK007 study, during the treatment phase 30 patients received a total of 49 infusions of MM-TK cells, with 17 patients (57%) receiving one infusion, 10 patients (33%) two infusions and 3 patients (10%) up to four infusions, which was the maximum number of infusions planned. The median number of MM-TK infusions was one (95% CI, 1 to 2) and the median cumulative cell dose administered was $1.1x10^7$ cells/kg (95% CI, 0.8 to 1.3). Furthermore, in this study 17 patients (57%) received fresh MM-TK cells and 13 subjects (43%) were administered cryopreserved MM-TK cells and 12 patients started with $1x10^6$/kg and 18 patients with $1x10^7$/kg. The first infusion was given at a median of 43 days after HSCT (range, 16 to 75) and the median interval time between the first and the subsequent infusions of MM-TK cells was 30 days (range, 27 to 39). Patients given cryopreserved cells received a lower number of infusions compared with those given fresh cells.

In the TK007 study protocol it is stated that in case of relapse, treatment could be started (as DLI) with a dose different from the first dose scheduled in the protocol, i.e. $1x10^7$/kg instead of $1x10^6$/kg.

In the follow-up phase, upon disease relapse or progression on study, 8 previously treated patients received an additional 16 infusions of MM-TK cells given as DLI. In detail, four patients had one infusion, one patient had two infusions, two patients had three infusions and one patient had four infusions. Since MM-TK administration in the setting of treatment of relapse (as DLI) was allowed, the maximal number of infusions of MM-TK with the purpose of IR, i.e. 4, could be and was exceeded. Also the planned dose of $1x10^7$/kg was exceeded for 5 patients (TK1, TK8, TK3, TK16, TK20) receiving MM-TK as DLI.

Regarding the ongoing phase II study TK008, during treatment phase, a total of 31 infusions of MM-TK cells were given to 15 patients, with five patients (33%) having one infusion, five patients (33%) two infusions, four patients (27%) three infusions and one (7%) four infusions, which was the maximum number of infusions planned by study protocol. The median number of MM-TK cell infusions per patient was two (95% CI, 1 to 3; range, 1 to 4), while the median cumulative cell dose was $2.4x10^7$/kg

(range, 1 to 3.9). The median time to first infusion was 28 days (95% CI, 25 to 30) after HSCT and the median interval time between the first and the subsequent cell doses administered was 32 days (95% CI, 30 to 40). The option to use MM-TK upon relapse as in TK007 was not allowed in TK008.

Adverse events

Secondary endpoints evaluating the safety of MM-TK included in the TK007 study that are discussed here are:

- Incidence of acute and chronic GvHD, diagnosed and graded according to published guidelines (Glucksberg, 1974 and Przepiorka, 1995 for acute disease; Filipovich, 2005 for chronic disease);
- Response to GCV used to activate the HSV-TK suicide gene;
- Acute and long-term toxicity related to MM-TK cells, with adverse events (AEs) graded according to National Cancer Institute Common Toxicity Criteria (NCI-CTC version 3.0);
- Vital signs, RCR test and monitoring of GvHD according to standard criteria.

Other (secondary) endpoints related to both efficacy and safety (non-relapse mortality, infectious events) are discussed in the efficacy section.

Analyses of AEs of the study TK007 included all AEs (coded according to MedDRA) occurred in safety population (n=52), which concerned all patients who received at least one haploidentical HSCT.

Common AEs

Overall, 603 AEs were reported and classified by severity grade as mild (8.6 %), moderate (23.9%), severe (34.5%), life-threatening (14.3%), no applicable grade (18.6%) and missing information (0.2%). Only 24 AEs (3.9%) were considered related to MM-TK cells and occurred in 11 of 30 treated patients (36.7%). According to the Applicant, most of the related AEs promptly resolved upon intervention. The AEs listed in this study include the AEs noted from the time of study enrolment. Thus, including the period before HSCT and well before first infusion of MM-TK. In addition, also all AEs seen in patients not receiving MM-TK (n=22) are incorporated in this listing.

The most common adverse events recorded in the study TK007 were infectious events. Viral infections accounted for 45% (112 of 249) of all infectious AEs and developed in 36 patients. A total of 87 cytomegalovirus (CMV) reactivations were reported in 32 high-risk seropositive patients and a total of 12 Epstein-Barr virus (EBV) infections were recorded in 10 patients. For treatment of CMV reactivation (val)ganciclovir is generally used. MM-TK cells are also sensitive to GCV. Present treatment of CMV is an exclusion criterion for MM-TK treatment, and MM-TK infusion must be performed 24 hrs after discontinuation of GCV according to the study protocol. In three patients a delay of MM-TK administration was recorded because of infection (CMV/EBV) requiring GCV treatment.

According to protocol, IL-2 should be administered at the 3rd and 4th dose. As it is known that IL-2 can have severe side effects, this could have affected the safety profile. However, the use of IL-2 in this study was limited. In the TK007 trial, only three patients (TK3-11-52) received the 3rd and the 4th MM-TK dose and only one patient (TK11) received IL-2, which was associated with grade 2 pancytopenia, grade 2 skin mass in the inoculum area and grade 1 nephropathy toxic at the third infusion, with the fourth IL-2 infusion being dose reduced by 50%. Two additional patients received two non-protocol-mandated IL-2 courses. Patient TK21 received an IL-2 course concomitantly with the second infusion of MM-TK cells following the physician's decision in order to intensify the graft versus leukaemia effect. Patient TK1 received an IL-2 course concomitantly with the fourth infusion of MM-TK given as donor lymphocyte infusion (DLI) for treating disease relapse. Both patients did not experience IL-2 related

adverse events. As IL-2 is not included in the treatment plan of the phase III trial TK008, further discussion on IL-2 is not deemed necessary.

The data indicate that apart from GvHD (discussed below), the MM-TK-related events appear not to be very specific as only 24 (4%) of the AEs were considered MM-TK related and occurred in 11 of the 30 (36.7%) treated patients (table 33). Notably, all AEs related to treatment were those that were (temporarily) associated with GvHD. This suggests in the opinion of the investigators, that GvHD is the main, or even, the only risk of MM-TK.

Table 15: Adverse events related to the MM-TK classified by Preferred Term and as worst grade per patient

Preferred term	Grade 1		Grade 2		Grade 3		Grade 4		NA		Total number of patients	
	No.	%	No.	%	No.	%	No.	%.	No.	%	No.	%
Acute GvHD	1	3.3	7	23.3	1	3.3	1	3.3	0	0.0	10	33.3
Pyrexia	1	3.3	0	0.0	0	0.0	1	3.3	0	0.0	2	6.7
Febrile neutropenia	0	0.0	0	0.0	1*	3.3	0	0.0	0	0.0	1	3.3
Intestinal haemorrhage	1*	3.3	0	0.0	0	0.0	0	0.0	0	0.0	1	3.3
Hepatic failure	0	0.0	0	0.0	1*	3.3	0	0.0	0	0.0	1	3.3
Chronic GvHD	0	0.0	0	0.0	0	0.0	0	0.0	1	3.3	1	3.3
Bronchitis	0	0.0	1	3.3	0	0.0	0	0.0	0	0.0	1	3.3
Haemoglobin decreased	0	0.0	1*	3.3	0	0.0	0	0.0	0	0.0	1	3.3
Platelet count decreased	0	0.0	0	0.0	0	0.0	1*	3.3	0	0.0	1	3.3
PTLD	0	0.0	0	0.0	1*	3.3	0	0.0	0	0.0	1	3.3

GvHD

The most common adverse event related to MM-TK cells was represented by GvHD. Among 30 treated patients, acute GvHD occurred in 10 patients (33%) with a median time to onset of 90 days (range, 20 to 162) after HSCT and 32 days (range, 8 to 91) after the last infusion of MM-TK cells.

GvHD is an expected complication/adverse event following allogeneic HSCT and/or infusion of allogeneic T-cells. Acute GvHD developed in 6 of 30 patients (20%) who initially received MM-TK cells during the treatment phase at a median of 94 days from HSTC, 48 days from first infusion of MM-TK cells and 17 days from immune reconstitution. Additionally, acute GvHD occurred in 4 of 8 patients (50%) who had received subsequent infusions of MM-TK cells given as DLI for treating disease relapse or progression at a median time of 21 days after last HSCT and 32 days after first infusion of MM-TK cells. None of these four patients had previously experienced MM-TK-related GvHD and none of other four retreated patients developed de novo GvHD.

The frequency of GvHD in TK007 is higher following MM-TK administered as DLI (4/8) as compared to the setting of MM-TK administration with the aim of IR (6/30), although it should be noted that the number of patients receiving MM-TK as DLI is very low. For the use of MM-TK as DLI, a higher cell dose/kg than planned was administered in 5/8 patients. This higher cell dose might, in part, explain the higher incidence of GvHD, as it is known that the risk for GvHD increases with increasing number of infused lymphocytes (as is also the case for MM-TK (see below)). Even so, the observed incidence of 20% for GvHD in TK007 during the treatment phase appears relatively low when compared to in the initially provided historical controls using DLI, where frequencies vary between 35% (Ciceri et al., 2008, Krishnamurthy et al., 2013) to 47% (Liga et al., 2013). However, interpreting this difference is

hampered by factors that influence the risk of developing GvHD, such as differences in the intensity of the conditioning regimens, the timing of DLI after allo-HSCT, the degree of HLA mismatch, the T-cell dose and subsets and the level of chimerism at time of DLI (Yun et al., 2013).

Safety data on GvHD from matched-pair analyses of MM-TK-group vs EBMT registry control population.

aGvHD

The percentage of patients developing Grade 2-4 GvHD seemed higher in the MM-TK treated group as compared to the control group, i.e. 35% vs 21%, respectively. When regarding Grade 3-4 aGvHD, there was no difference between the groups, 8% vs 9%, respectively. For both parameters, the differences were not statistically significant. Even so, when correlating the development of aGvHD with clinical outcome parameters, the data showed a positive effect of developing acute GvHD in MM-TK patients versus control patients on NRM, OS and LFS. This implies that the development of acute GvHD upon MM-TK is associated with an anti-leukaemia effect. However, relevance of these results is limited as the aGvHD patient numbers involved are low (n=29 in the control group and n=13 in the MM-TK-group), the patients that are at risk developing aGvHD upon MM-TK treatment cannot be identified upfront and the anti-leukaemic effect (as measured in RI and LFS) was not observed in the overall patient comparison.

cGVHD

Chronic GvHD was graded according to the revised Seattle criteria. In the matched-pair, a difference in favour of the MM-TK-group could be observed for the 1-year incidence of cGvHD with 28% for the control group vs 7% for the MM-TK-group (p=0.04). These data can be considered of interest. The mechanism behind this difference is not completely understood. An analysis on potential baseline characteristics that may have affected the incidence of cGvHD did not identify a baseline parameters that could explain the observed difference in cGvHD incidence.

Treatment of GvHD

For treating (acute)GvHD, the suicide gene was activated by administering ganciclovir that is able to selectively eliminate the HSV-TK genetically-modified cells. In TK007, for treating MM-TK-related GvHD, four patients received ganciclovir intravenously and six patients valganciclovir orally. In seven patients it was needed to add a standard immunosuppressive treatment, but the acute GvHD was fully controlled in all the ten treated patients by ganciclovir/valganciclovir alone (n=3) or in combination with corticosteroids (CS) (n=5) or in combination with corticosteroids and mycophenolate mofetil (n=1) or in combination with corticosteroids, mycophenolate mofetil and cyclosporine (n=1). One patient with grade 1 did not require treatment (see table 34).

According to the Applicant patients received 25-50% of the CS dose normally recommended for grade 2 to 4 acute GvHD. In 3 patients CS was given before GCV treatment, at/after onset of GvHD, which is not according to protocol. As in these 3 patients an effect of GCV treatment on LNGFR+ cells is seen (see table 34), and CS treatment was approximately 0.5 mg/kg/day, it is likely that GCV treatment indeed contributed to the resolution of GvHD.

Table 16: List of patients with GvHD related to MM-TK cells and timing of treatments

Pat	GvHD					Ganciclovir			Corticosteroids			Mycophenolate		
	Gr			Days	Out come			Days			Days			Days
TK5	2			22	CR			15			56			-
TK8	4			53	CR			14			53			15
TK16	2			7	CR			14			12			-
TK20	2			44	CR			49			4			
TK25	2			10	CR			16			22			
TK38	3			12	CR			15			-			
TK43	2			12	CR			10			-			-
TK44	ch			107	CR			15			-			123
TK47	2			10	CR			18			56			-
TK50	2			21	CR			16			-			-

^ Topical (eye drops), ch=chronic

According to the Applicant, all acute GvHD events fully resolved after a median duration of 12 days (range, 7 to 64). Only one patient (3%) developed extensive chronic GvHD that occurred 159 days and 129 days after HSCT and last infusion, respectively, and fully resolved after 107 days. In these 10 ganciclovir-treated patients who suffered grade 2 to 4 acute or chronic GvHD, absolute LNGFR+ counts, as enumerated by flow cytometric analysis or by PCR, significantly decreased during ganciclovir treatment, either at 4 days after onset of GCV treatment and/or post GCV treatment (see table 35). The reduction in MM-TK cells following GCV treatment indicates that the transgene is active, and GCV exposure does induce the suicide of thymidine kinase positive cells. What is also noted is that GCV does not kill all MM-TK cells upon treatment. Importantly, the majority of the patients receiving GCV treatment for GvHD also received additional medication.

Table 17: Reduction of LNGFR+ and MM-TK+ cells in GvHD patients treated with GCV

Pat. Nr.	GvHD			GCV	LNGFR$^+$ (cells/µL)			MM-TK$^+$ (%)			GvHD out come
	TK$^+$ cells/kg	Gr	Dur. (days)	Dur. (days)	Pre GCV	4th day	Post GCV	Pre GCV	4th day	Post GCV	
TK5	1.1x10^7	2	22	15	36	10	7	6	0.5	0.3	CR
TK38	1.3x10^7	3	12	15	99	48	6	4.8	0.1	0.9	CR
TK43	0.1x10^7	2	12	10	311		30	15.7	9.8	1.6	CR
TK47	1.3x10^7	2	10	18	221		34	25.8	9.3	4.3	CR
TK50	1.3x10^7	2	21	16	335	151	26	37.6	9.4	4.5	CR
TK44	0.1x10^7	chr	107	15	12	30	0	1	1	1	CR
TK8	10x10^7	4	53	14	308	181	24	30	7.6	6.8	CR
TK16	2.2x10^7	2	7	14	90	23	111	3.2	0.6	2.6	CR
TK20	2.4x10^7	2	44	49	414	166	62	16.2	6.1	0.2	CR
TK25	0.4x107	2	10	16	22	13	27	4	0.5	0.8	CR

Circulating LNGFR+ cells

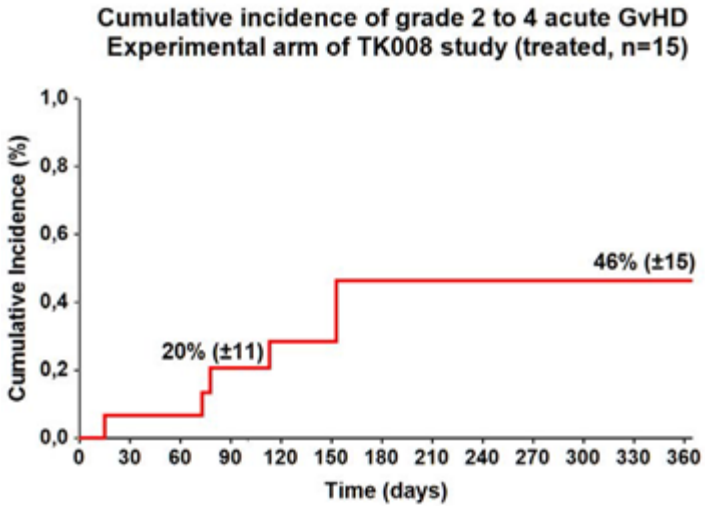
The data as provided by the Applicant on circulating LNGFR+ cells suggest that a high engraftment of MM-TK cells predisposes for developing GvHD. This observation, together with the fact that there was no significant difference in CD3+ cells observed in patients with or without GvHD, indicates that, in general, GvHD is caused by the MM-TK cells and not by the T-cells originating from the HSC graft. However, it should be noted that conclusion is based on observations in only 11 patients with GvHD and 12 patients without GvHD, who all achieved IR.

Preliminary results on GvHD in TK008

Among 15 treated patients, acute GvHD developed in 8 patients (53%) with a median time to onset of 115 days (range, 15 to 181) after HSCT and 31 days (range, 10 to 89) after last infusion of MM-TK cells. Severity of acute GvHD was grade 1 in two cases (13%), grade 2 in five (33%) and grade 3 in one (7%). All acute GvHD events fully resolved after a median duration of 17 days (95% CI, 7 to 29; range, 6 to 29). There were no grade 4 events or GvHD-related deaths or long-term complications.

Cumulative incidence rates of grade 2 to 4 acute GvHD related to MM-TK cells was 20% (±11%) at 100 days and 46% (±15%) at 1 year (Figure 18).

Figure 2: TK008: Cumulative incidence of grade 2 to 4 acute GvHD related to MM-TK cells



Considering these data, the frequency of GvHD appears higher in TK008 than seen in TK007. Possible explanations for this difference may be 1) the higher cell dose that was administered per kg per patients in TK008 as compared to TK007 and/or 2) the different composition of the MM-TK cell suspension between the 2 studies (be referred to the quality section for details). It is not clear to what extent these aspects contributed to the incidence of GvHD. Furthermore, no grade 4 GvHD was observed and GvHD was apparently treatable as all cases resolved.

For treating MM-TK-related GvHD, patients received ganciclovir intravenously or valganciclovir orally. Two patients with grade 1 acute GvHD did not receive any treatment. All signs and symptoms of grade 2 to 4 acute GvHD fully resolved after a median treatment duration of ganciclovir or valganciclovir of 14 days (95% CI, 9 to 34) (table 36).

Table 18: TK008: List of patients with GvHD related to MM-TK cells and response to ganciclovir

Pat. Nr.	No. of infusions	GvHD					Ganciclovir treatment	GvHD outcome
		MM-TK$^+$ cells/kg	Days after HSCT	Days after infusion	Grade	Duration (days)	Duration (days)	
TK7A	2	$2.4x10^7$	117	30	1	20	-^	CR
TK10A	3	$3.6x10^7$	153	19	2	29	34	CR
TK11A	2	$2.2x10^7$	113	55	2	7	14	CR
TK12A	1	$1.3x10^7$	37	33	3	9	9	CR
TK13A	4	$3.6x10^7$	153	13	2	14	15	CR
TK14A	1	$1.0x10^7$	15	10	2	6	10	CR
TK15A	2	$2.6x10^7$	181	89	1	21	-^	CR
TK17A	1	$1.4x10^7$	73	37	2	21	15	CR

Serious adverse events and deaths

SAEs

In study TK007, a total of 118 SAEs were registered in 46 of 52 (88%) subjects included in the safety population. Among these, 88 (75%) occurred in 27/30 (90%) patients who were treated with MM-TK cells. Only two SAEs (acute GvHD in 2 patients) were considered related to MM-TK cells. SAEs very common in frequency were represented by cytomegalovirus infection (42%), leukaemia recurrent

(21%) and pneumonia (12%). This pattern of most common SAEs is not unexpected considering the population and the treatments at hand.

Deaths

Among the 52 patients in the TK007 safety population, 43 (83%) died during the TK007 study. Twenty-two deaths (73%) occurred among the 30 patients treated with MM-TK cells and 21 (95%) among the 22 patients who were untreated with MM-TK cells. The main causes of death reported by investigators were infection (32.6%), multi-organ failure (21.0%), leukaemia recurrent (16.3%), disease progression (9.3%), acute respiratory distress syndrome (7.0%), acute myocardial infarction, cerebral haemorrhage, encephalitis by cytomegalovirus, hepatic failure, transplant failure and unknown cause (2.3% for each cause). In TK008, among the 17 patients who were randomly assigned to arm A, three died without previous occurrence of disease relapse or progression: one patient (TK4A) who did not receive MM-TK cells died of sepsis, one of cerebral haemorrhage (TK7A) and one (TK12A) of multi-organ failure. The Applicant classified these deaths as not related to MM-TK cells. It is noted that two cases of cerebral haemorrhage were reported (1 in TK007 and 1 in TK008). Upon discussion of these cases, it is agreed with the Applicant that these cerebral haemorrhage events are likely to be unrelated to MM-TK administration.

Importantly, the high frequency of death on study (in TK007 and TK008) can be seen to support the statement that MM-TK is intended for a life-threatening condition for which there is an unmet medical need.

In study TK007 there was a high rate of graft failure/rejection (23%). In the TK008 study no case was reported until now. There is no convincing explanation for this difference. It may, in part be due to chance, however also the higher proportion of poor-prognosis cases enrolled in TK007 than TK008 could have played a role.

Vital signs, physical findings and other observations related to safety

No clinically significant modifications of vital signs or physical findings related to MM-TK cells were reported except for pyrexia experienced by two patients (TK8 and TK16) during the study. Of one of these patients pyrexia was mild in severity, but life-threatening in the second one. In both patients the fever lasted 5 days and occurred 19 and 9 days after last DLI, respectively. Both patients were successfully treated.

Laboratory findings

No clinically significant modifications related to MM-TK cells were reported in the laboratory examinations. Only one patient had concomitant febrile neutropenia, haemoglobin decrease and platelet count decrease considered related to MM-TK.

RCR: The RCR search on genomic DNA from PBMCs was carried out before and after each MM-TK infusion, and at 3, 6 and 12 months after first infusion. All tested samples were negative.

Safety in special populations

No studies were performed in special populations.

No discrimination was made regarding the age of the patients, which is acceptable as only 2 patients were ≥ 65years, i.e. (TK35 (66y) en TK37 (65y)), and considering the overall small size of the study.

Immunological events

This aspect was not discussed by the Applicant. In principle, there is no specific cause for concern for potential immunological events related to the MM-TK cells as these cells are from the HSC donor. However, two transgenes are inserted and expressed, i.e. the HSV-TK and ΔLNGFR. In principle these

may activate the immune system. However, the patients are (severely) immune suppressed at the time of MM-TK infusion, making this a theoretical concern during the period of immune-suppression. This should be included in the RMP as missing information.

Safety related to drug-drug interactions and other interactions

No information/discussion was provided by the Applicant on this aspect. Possible interaction with antiviral therapy has been discussed above.

Discontinuation due to AES

Six patients (20%) experienced a total of eight AEs that led to a dose modification of MM-TK cells. Five patients (TK11-38-47-52-54) had a delay of MM-TK infusions (two of first infusion and four of the subsequent infusions), while one patient (TK18) required a higher dose ($3x10^7$/kg) because of concomitant CMV and EBV infections. From the information provided by the Applicant it is understood that these modifications were not due to AES related to MM-TK, but due to treatment of CMV infection with GCV, graft rejection, lymphoid chimerism or AEs caused by IL-2.

Of the 52 patients included in the study patients, 22 did not receive MM-TK due to early death (n=12), graft failure/rejection (n=7), prolonged administration of ganciclovir or immunosuppressive therapy after HSCT (n=3).

2.6.1. Discussion on clinical safety

Patients are exposed to MM-TK within the context of two clinical trials, i.e. TK007 (completed) and TK008 (ongoing), and the number of patients is limited. Information on the safety is primarily based on the 52 patients included in trial TK007 of whom during the treatment phase 30 patients received a total of 49 infusions of MM-TK cells. Twenty-two patients did not receive MM-TK cells due to early death (n=12), graft failure/rejection (n=7; 6 patients were retransplanted) and prolonged administration of ganciclovir or immunosuppressive therapy after HSCT (n=3). The median number of MM-TK infusions was 1 (95% CI, 1 to 2) and the median cumulative cell dose administered was $1.1x10^7$ cells/kg (95% CI, 0.8 to 1.3). In the follow-up phase, following disease relapse or progression on study, 8 previously treated patients received an additional 16 infusions of MM-TK cells as DLI. Preliminary TK008 safety data, on GvHD only, have been provided for 15 patients in the experimental arm (see section on S*upportive studies*).

Adverse events

Overall, 603 AEs were reported and classified by severity grade as mild (8.6%), moderate (23.9%), severe (34.5%), life-threatening (14.3%), no applicable grade (18.6%) and missing information (0.2%). The most common AEs recorded in the study TK007 were represented by infectious events, including a total of 87 CMV reactivations. For treatment of CMV reactivation (val)ganciclovir (GCV) is generally used, however also MM-TK cells are sensitive to GCV. Because of this, a 24hr wash-out period was recommended. According to protocol, IL-2 should be administered at the 3rd and 4th MM-TK dose. As it is known that IL-2 can have severe side effects, this could have affected the safety profile. However, the use of IL-2 in this study was limited. Indeed, of the 3 patients receiving a 3rd and 4th dose, only one received concomitant Il-2, which was associated with IL-2-related AEs. Two additional patients received two non-protocol-mandated IL-2 courses, and these patients did not appear to experience IL-2 related adverse events. As IL-2 is not included in the treatment plan of the phase III trial TK008, further discussion on IL-2 is not deemed necessary.

Only 24 AEs (3.9%) were considered related to MM-TK cells and occurred in 11 of 30 treated patients (36.7%). All AEs related to treatment were those that were (temporarily) associated with GvHD. This

suggests that in the opinion of the investigators, GvHD is the main, or even only, risk of MM-TK. Apart from GvHD the profile of the other MM-TK-related AE (pyrexia, febrile neutropenia, hepatic failure, bronchitis, decreased haemoglobin, post-transplant lymphoproliferative disorder) appears not to be very specific. Most of the related AEs promptly resolved with appropriate medical intervention.

Graft versus host disease (GvHD) is an expected complication/adverse event following allogeneic HSCT and/or infusion of allogeneic T-cells. In TK007, acute GvHD developed in 6 of 30 patients (20%) who received MM-TK cells during the initial treatment phase at a median of 94 days from HSTC, 48 days from first infusion of MM-TK cells and 17 days from immune reconstitution. Additionally, acute GvHD occurred in 4 of 8 patients (50%) who had received subsequent infusions of MM-TK cells given as DLI for treating disease relapse or progression at a median time of 21 days after last HSCT and 32 days after first infusion of MM-TK cells. The observed incidence of 20% for GvHD during the treatment phase appears relatively low when compared to historical controls using DLI, however, interpreting this difference is hampered by donor- and treatment-related factors that influence the risk of developing GvHD.

Patients who developed MM-TK-related GvHD (n=11, 10 acute GvHD and 1 chronic GvHD) exhibited at the time of IR and at peak cell levels, more LNGFR+ cells than patients who did not develop GvHD (n=12). This suggests that a higher engraftment/exposure to MM-TK cells correlates to developing GvHD. There were no significant differences in CD3+ cells in patients with or without GvHD, not at the time of IR and not at time of the peak level of CD3+ cells. Together, this suggests that GvHD is caused by MM-TK cells and not by the T-cells originating from the HSC graft. Cautionary note: this is based on observations in a low number of patients.

Ten patients suffering from grade 2 to 4 acute or chronic GvHD were treated with (val)ganciclovir to activate the suicide gene in the MM-TK cells. As a result in all patients, the number of circulating LNFGR+ cells (i.e. MM-TK cells) was reduced.

Despite the fact that the level of circulating MM-TK cells seem to predispose for GvHD, and that circulating cells were reduced upon treatment in most of the patients, 7 of the 10 patients receiving GCV for GvHD received additional immune-suppressive therapy to control the GvHD, mostly corticosteroids. According to the Applicant patients received 25-50% of the dose normally recommended for grade 2 to 4 acute GvHD. Based on the provided data, it cannot be excluded that the corticosteroids were given to the patients to prevent (further) development of GvHD. However, as GCV treatment reduced the number of LNGFR+ cells, it is not likely that the concomitant use of corticosteroids meaningfully interfered with the resolution of MM-TK-induced GvHD by GCV.

A difference in favour of the MM-TK-group could be observed for the 1-year incidence of chronic GvHD (28% vs 7%, p=0.04). The Applicant suggested that control by the suicide system plays a role in the reduced chronic GvHD incidence. However, it not evident that the same mechanism described above is involved in all of the chronic GvHD cases. At the time of onset of chronic GvHD, T cells emanating from the stem cell graft outnumber the circulating MM-TK cells, and thus it may be more likely that chronic GvHD is caused by these stem cell graft- derived T cells instead of by the MM-TK cells. Therefore, the observed beneficial effect of MM-TK on cGvHD-incidence remains unexplained. The provided analysis on potential baseline characteristics did not reveal an alternative explanation for the observed difference in cGvHD incidence other than MM-TK treatment. As chronic GvHD is associated with late (beyond 1 year post HSCT) non-relapse mortality (Boyiadzis 2014), this reduction in incidence might not only consist of a reduction in morbidity but also in mortality. However as patients numbers beyond 1 year are low no meaningful conclusion can be drawn on this issue.

Dose modifications

Six patients (20%) experienced a total of eight AEs that led to a dose modification (delay (n=5), and higher dose (n=1)) of MM-TK cells. From the information it is understood that these modifications were not due to AES related to MM-TK,

The occurrence of replication-competent retrovirus (RCR) was studied according to protocol. All, patient samples were negative.

Additional safety data needed in the context of a conditional MA

In order to confirm the efficacy and safety of Zalmoxis as an adjunctive treatment in haploidentical haematopoietic stem-cell transplantation of adult patients with high-risk haematological malignancies, the MAH should submit the results of study TK008, a randomized phase III trial of haploidentical HCT with or without an add back strategy of HSV-Tk donor lymphocytes in patients with high risk acute leukaemia.

The MAH will follow-up on the actual use and effectiveness of the product in clinical practice.

In addition, the MAH will follow-up on the risks of Graft versus Host Disease (GvHD), Severe systemic infection CMV and EBV reactivation, Febrile neutropenia, Hepatic failure, Carcinogenicity, Genotoxicity, Late complications (malignancies, autoimmunity), Immunological events (antibody formation), Use in paediatric patients, Use in pregnant women, Use in breast feeding women, Use in patients with renal impairment, Use in patients with hepatic impairment and Use in elderly.

This data will be collected within through a non-Interventional PASS (study TK011) in order to investigate the safety and the effectiveness of Zalmoxis in real clinical practice as well as long-term safety and efficacy. In the context of this study, the MAH should collect data about the disease status and outcome of all patients treated with Zalmoxis using the EBMT registry.

2.6.2. Conclusions on the clinical safety

The safety database for this product is very small, 30 patients receiving MM-TK in a completed trial, and an additional 15 patients in an ongoing trial with incomplete follow up and of whom only limited information was provided. The results showed that the most common AE was infection and the most common treatment-related AE was acute GvHD. Overall, the aGvHD appeared to be treatable, with all cases attributed to MM-TK resolved. Regarding the concomitant use of corticosteroids, it is not likely that concomitant use of corticosteroids affects the activity of GCV regarding the resolution of MM-TK-induced aGvHD. Yet, considering that, though pointing in the right direction, only limited data are available on the concomitant use of immunosuppressive agents, additional information on this subject is awaited from the specific obligation (TK008) in the context of a conditional MA.

Furthermore, based on the available information on the treatment of CMV infection, on the treatment of GvHD and the pharmacodynamic interaction of GCV with the MM-TK cells, it is recommended that the Applicant systematically reports by means of regular pharmacovigilance activities on the type and the efficacy (response and duration) of the treatment of CMV reactivation. Finally, considering the above, the safety data from the phase III trial would be highly welcomed. From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.
The CAT considers the following measures necessary to further confirm the benefit-risk of Zalmoxis, in the context of a conditional MA:

In order to confirm the efficacy and safety of Zalmoxis as an adjunctive treatment in haploidentical haematopoietic stem-cell transplantation of adult patients with high-risk haematological malignancies, the MAH should submit the results of study TK008, a randomized phase III trial of haploidentical HCT

with or without an add back strategy of HSV-Tk donor lymphocytes in patients with high risk acute leukaemia.

The CAT considers the following measures necessary to address issues related to safety and the effectiveness of the product:

Non Interventional safety and efficacy study (TK011): In order to investigate the safety and the effectiveness of Zalmoxis in real clinical practice as well as long-term safety and effectiveness, the MAH should conduct a study to collect data about the disease status and outcome of all patients treated with Zalmoxis using the EBMT registry.

The CHMP endorse the CAT conclusion on clinical safety as described above.

2.7. Risk Management Plan

The CAT received the following PRAC Advice on the submitted Risk Management Plan (RMP):

The PRAC considered that the RMP version 1.0 (dated (22 February 2014) could be acceptable if the Applicant implements the changes to the RMP as described in the PRAC endorsed PRAC Rapporteur assessment report dated 10 July 2014.

The CAT endorsed this advice.

The Applicant implemented all changes to the RMP as requested by the PRAC and the CAT.

The CAT endorsed the RMP version 6.1 (dated 22 June 2016) with the following content:

Safety concerns

Table 19: Summary of the safety concerns

Important identified risks	• Graft versus Host Disease (GvHD) • Sever systemic infection • Cytomegalovirus (CMV) and Epstein-Barr Virus (EBV) reactivation • Febrile neutropenia • Hepatic failure • Concomitant administration of ganciclovir or valganciclovir

Important potential risks	• Concomitant immunosuppressive therapy • Development of Replication Competent Retrovirus (RCR) • Carcinogenicity • Genotoxicity • Late complications (malignancies, autoimmunity) • Immunological events (antibody formation) • DMSO-related side effects • Donor site reaction local • Donor reaction systemic • Treatment failure (GvHD not treatable with GCV)
Missing information	• Use in paediatric patients • Use in pregnant women • Use in breast feeding women • Use in patients with renal impairment • Use in patients with hepatic impairment • Use in elderly patients

Pharmacovigilance plan

Table 20: Ongoing and planned additional PhV studies/activities in the Pharmacovigilance Plan

Study / activity Type, title and category (1-3)	Objectives	Safety concerns addressed	Status (planned or started)	Date for submission of interim or final reports (planned or actual)
PIP trial TK009, interventional clinical study, (category 3)	Assess safety in paediatric patients from birth to less than 18 years	Use in paediatric patients from birth to less than 18 years	Deferred	Date of completion, December 2018, deferred
PIP trial TK010, interventional clinical study, (category 3)	Assess safety in paediatric patients from birth to less than 18 years	Use in paediatric patients from birth to less than 18 years	Deferred	Date of completion, December 2022, deferred

Study / activity Type, title and category (1-3)	Objectives	Safety concerns addressed	Status (planned or started)	Date for submission of interim or final reports (planned or actual)
Study TK008 (category 2)	Phase III randomised clinical trial: in order to confirm the efficacy and safety of Zalmoxis as an adjunctive treatment in haploidentical haematopoietic stem-cell transplantation of adult patients with high-risk haematological malignancies, the MAH should submit the results of study TK008, a randomized phase III trial of haploidentical HCT with an add back strategy of HSV-Tk donor lymphocytes in patients with high risk acute leukaemia.	Graft versus Host Disease (GvHD) Severe systemic infection Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) reactivation Febrile neutropenia Hepatic failure Concomitant administration of ganciclovir or valganciclovir Concomitant immunosuppressive therapy Development of Replication Competent Retrovirus (RCR) Carcinogenicity Genotoxicity Late complications (malignancies, autoimmunity) Immunological events (antibody formation) DMSO-related side effects Donor site reaction local Donor reaction systemic Treatment failure (GvHD not treatable with GCV) Use in paediatric patients, Use in pregnant women, Use in breast feeding women, Use in patients with renal impairment, Use in patients with hepatic impairment Use in elderly patients	Renewal pf the conditional marketing authorisation Periodic update on recruitment (included in the PSUR) Date of accrual end Follow up: 1 year Final Clinical Study Report	Every year Every 6 months March 2019 March 2020 March 2021

Study / activity Type, title and category (1-3)	Objectives	Safety concerns addressed	Status (planned or started)	Date for submission of interim or final reports (planned or actual)
TK011 PASS (category 1)	Non Interventional PASS: In order to investigate the safety and effectiveness in real clinical practice as well as long-term safety and effectiveness in all patients treated with Zalmoxis, the MAH should conduct and submit the results of study TK011 using the EBMT registry including the patients treated with Zalmoxis. **At least 30 patients for each group are expected to be accrued per year**.	Graft versus host disease (GvHD) Severe systemic infection Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) reactivation Febrile neutropenia Hepatic failure Concomitant administration of ganciclovir or valganciclovir Concomitant immunosuppressive therapy Development of Replication Competent Retrovirus (RCR) Carcinogenicity Genotoxicity Late complications (malignancies, autoimmunity) Immunological events (antibody formation) DMSO-related side effects Donor site reaction local Donor site reaction systemic Treatment failure (GvHD not treatable with GCV) Use in paediatric patients Use in pregnant women Use in breast feeding women Use in patients with renal impairment Use in patients with hepatic impairment Use in elderly patients	Updated study protocol Registration in the EU PAS Register Start of data collection End of data collection Study progress report 1* Study progress report 2* Study progress report 3* Study progress report 4* Final Clinical study report	To be submitted no later than 6 months after the granting of the MA 2016 2017 2021 2018* 2019* 2020* 2021* By Q4 2022

***Yearly evaluation of safety and effectiveness data (submission of data to be synchronised with the annual renewal of the conditional marketing authorization)**

Risk minimisation measures

Table 21: Summary table of Risk Minimisation Measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Graft versus Host Disease (GvHD)	Listed as undesirable effect in section 4.8 of the SmPC and section 4 of the PIL Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients .	Educational material
Severe systemic infection	Listed as undesirable effect ("bronchitis") in section 4.8 of the SmPC and section 4 of the PIL Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
CMV and EBV reactivation	Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
Febrile neutropenia	Listed as undesirable effect in section 4.8 of the SmPC and section 4 of the PIL Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
Hepatic failure	A Listed as undesirable effect in section 4.8 of the SmPC and section 4 of the PIL Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
Concomitant administration of ganciclovir or valganciclovir	A warning is placed in section 4.4 of the SmPC and section 2 of the PIL: Patients should not be administered if the following conditions occurs: a) infections requiring administration of ganciclovir or valganciclovir at the time of infusion; Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	Educational material

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Concomitant immunosuppressive therapy	A warning is placed in section 4.4 of the SmPC and section 2 of the PIL: Patients should not be administered if the following conditions occurs: c) ongoing systemic immunosuppressive therapy or administration of granulocyte colony stimulating factor (G-CSF) after haploidentical hematopoietic stem-cell transplantation; Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	Educational material
Development of Replication Competent Retrovirus (RCR)	Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
Carcinogenicity	Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
Genotoxicity	Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
Late complications (malignancies, autoimmunity)	Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
Immunological events (antibody formation)	Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
DMSO-related side effects	Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
Donor site reaction local	Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
Donor reaction systemic	Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Treatment failure (GvHD not treatable with GCV)	Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
Use in paediatric patients	A warning is placed in section 4.2 of the SmPC and section 2 of the PIL: The safety and efficacy in children and adolescents (less than 18 years) have not been established. No data are available. Zalmoxis is therefore not recommended for use in children and adolescent below 18 years. A text is placed in section 4.8 of the SmPC: No specific paediatric group has been studied at present. Only one 17-year-old male, affected by T lymphoblastic lymphoma, was treated in the TK007 trial with two infusions of Zalmoxis. No adverse reactions were reported for this patient. Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
Use in pregnant women	Use of MM-TK is contraindicated in pregnant women, SmPC 4.3. A warning is placed in section 4.6 of the SmPC and section 2 of the PIL: There are no data from the use of Zalmoxis in pregnant women. Animal studies are insufficient with respect to reproductive toxicity. Zalmoxis is not recommended during pregnancy and in women of childbearing potential not using contraception (see section 4.3). Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Use in breast feeding women	Use of MM-TK is contraindicated in breastfeeding women, SmPC 4.3. A warning is placed in section 4.6 of the SmPC and section 2 of the PIL: There is insufficient information on the excretion of Zalmoxis/metabolites in human milk. Zalmoxis should not be used during breast-feeding (see section 4.3). Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
Use in patients with renal impairment	Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
Use in patients with hepatic impairment	Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None
Use in elderly patients	A text is placed in section 4.8 of the SmPC: In the TK007 clinical study only one 66-year-old female was treated with one infusion of Zalmoxis. The patient did not experience any adverse reaction. No implications on the use of Zalmoxis in patients of 65 and older have been established. Patient labelled prescription only medicinal product. To be used under the supervision of health care providers experienced in the treatment of HSCT patients.	None

The CHMP endorse the PRAC and CAT advice on the RMP.

2.8. Pharmacovigilance

Pharmacovigilance system

The CAT considered that the pharmacovigilance system submitted by the applicant fulfils the requirements of Article 8(3)(ia) of Directive 2001/83/EC.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Zalmoxis is included in the additional monitoring list as a medicinal product authorised in the Union that contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the Union (Article 23(1)(a)); and medicinal product that is authorised pursuant to this Regulation, subject to the conditions referred to in Article 14(7) and Article 9(4)(cb) of Regulation (EC) no 726/2004.(Article 23(1)(c)).

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

Benefits

Beneficial effects

The results from matched-pair analyses in 47 patients treated with MM-TK cells (n=30 in TK007 and n=17 in TK008) matched with 140 controls from the EBMT database showed that the MM-TK-treated patients seem to benefit in terms of 1 year OS (i.e. median 49% for MM-TK vs 37% for control, p=0.01) and 1-year NRM (i.e. median 22% for MM-TK vs median 43% for control, p=0.014), but that there is no advantage in 1-year LFS (i.e. median 37% for MM-TK vs median 35% for control) and the chance for relapse (i.e. median 41% for MM-TK vs median 22% for control), because NRM and relapse are competing risk events and relapse events occur later than NRM events.

The data from the landmark analysis which excluded patients who either died, relapsed or experiencing early graft failure (lack of myeloid engraftment) before day 21, show that the MM-TK treated patients that had survived the first 3 weeks post-transplant benefited in terms of 1 year OS (40% vs 51% (p=0.03)) and 1-year NRM (42% vs 23% (p=0.04)), for control vs MM-TK treated patients, respectively. Also here there was no significant difference regarding LFS and the chance for relapse.

In an alternative analysis aiming to decrease the uncertainty regarding the adequate matching of patients for early post HSCT events, MM-TK and control patients who were alive and relapse-free were matched at the 21 day post HSCT time point. The same matching parameters were used as for the initial matched-pair analysis. In this analysis 139 control patients (70 T-cell replete and 69 T-cell depleted grafts) were matched with 36 TK-treated patients. The data from this analysis an absolute increase of 17% in 1-year OS rate for the MM-TK group compared with the control group (51% vs 34%; p=0.007) and an a benefit in 1-year NRM (20% vs 46%; p=0.003) for MM-TK treated patients. Again, also here no significant difference regarding LFS was observed while chance for relapse seemed higher in the MM-TK-treated patients as compared to the control patients. Notably, relapse and NRM are competing risks.

In order to minimize bias due to differences in the timing of MM-TK infusion, 3 further 'sensitivity' analyses were performed in which patients were excluded who had died or relapsed before 4, 6 and 8 weeks after transplant. These analyses revealed similar significant improvements in OS, NRM and chronic GvHD in favour of MM-TK-treated subjects as compared to control patients as seen with the analysis using the 3 week time point. Also the difference in relapse incidence and leukaemia free survival did not appear to change substantially.

Uncertainty in the knowledge about the beneficial effects

Data from an adequately controlled trial are lacking, and all data on the efficacy of MM-TK treatment comes from a cross study comparison using a historical control group. Thus all efficacy data are associated with the uncertainties inherent to cross study comparisons. The main uncertainties associated with the current comparison pertain to the low number of patients, the unknown impact of potential differences in baseline characteristics not included in the matching strategy (e.g. median year of transplant, stem cell source, conditioning regimen) and the inherent uncertainty with cross study comparison. The role of MM-TK cells to a (hastened) IR cannot formally be established. However, considering the temporal association between the administration of MM-TK and occurrence of IR, it is likely that MM-TK has contributed. Even though the results are suggestive of IR being an early surrogate marker for efficacy in the small uncontrolled TK007 study in terms of OS, infections and reduced NRM, this is not based on a comparison between randomised groups and thus firm conclusions cannot be drawn. Results from the confirmatory study TK008 are expected to address the above uncertainties (see Annex II and RMP).

The long-term efficacy remains uncertain as data are very limited. However this may be accepted as NRM is the main driver of the effect of MM-TK on OS, and NRM generally occurs within the first year post transplant. Long term data will be provided through the regular reporting from EBMT in study TK011 (see Annex II and RMP).

Risks

Unfavourable effects

Of all 603 AEs reported in TK007, only 24 AEs (3.9%) were considered related to MM-TK cells and occurred in 11 of 30 treated patients (36.7%). All the related AEs were reported to be associated with GvHD and most promptly resolved upon intervention. This suggests that GvHD can be regarded as the the main, or even, the only risk of MM-TK. In TK007, among 30 treated patients, acute GvHD occurred in 10 patients (33%). MM-TK-related GvHD, was treated with iv GCV in 4 patients and oral valganciclovir in 6 patients. In all the ten treated patients, acute GvHD was fully controlled by GCV/valganciclovir alone (n=3) or in combination with corticosteroids (CS) (n=5) or in combination with CS and mycophenolate mofetil (n=1) or in combination with CS, mycophenolate mofetil and cyclosporine (n=1). One patient with grade 1 GvHD did not require treatment.

Only 2 out of a total of 118 SAEs (acute GvHD in 2 patients) were considered related to MM-TK cells. None of the deaths in both TK007 and TK008 studies were attributed to MM-TK treatment.

Six patients (20%) experienced a total of eight AEs that led to a dose modification of MM-TK cells. Five patients had a delay of MM-TK infusions, while one patient (TK18) required a higher dose ($3x10^7$/Kg), because of concomitant CMV and EBV infections.

The preliminary results from TK008 showed that among 15 treated patients, acute GvHD developed in eight patients (53%). The 6 patients experiencing grade 2 to 4 GvHD were treated with GCV alone (n=2) or in combination with CS (n=4). All acute GvHD events fully resolved after a median duration of 17 days (95% CI, 7 to 29; range, 6 to 29) of which the grade 2 to 4 acute GvHD (n=6) fully resolved after a median treatment duration of ganciclovir or valganciclovir of 14 days (95% CI, 9 to

34). These data show that the incidence of GvHD appears higher in TK008 than in TK007. Regarding the matched-pair analyses, the percentage of patients developing Grade 2-4 GvHD seemed higher in the MM-TK treated group as compared to the control group, i.e. 35% vs 21%, respectively. When regarding Grade 3-4 aGvHD, there was no difference between the groups, 8% vs 9%, respectively. For both parameters, the differences were not statistically significant. When correlating the development of aGvHD with clinical outcome parameters, the data showed a positive effect of developing acute GvHD in MM-TK patients versus control patients on NRM, OS and LFS.

The 1-year incidence of chronic GvHD was lower in the MM-TK group when compared to controls, this was observed across the matched-pair analyses (e.g. 23% vs 6%; p=0.02, matched-pair at day 21 post HSCT analysis).

Uncertainty in the knowledge about the unfavourable effects

The observed incidence of 20% for acute GvHD during the treatment phase in TK007 is relatively low as compared to historical DLI controls (administered in relapse settings), that varied between 35% (Ciceri et al., 2008; Krishnamurthy et al., 2013) and 47% (Liga et al., 2013). However, the TK007 results are obtained with genetically modified lymphocytes and based on a limited number of GvHD (n=11)/non-GvHD (n=12) patients within the patient group achieving IR upon MM-TK (n=23). Furthermore, variables should be taken into account that influence the risk of developing GvHD, e.g. the intensity of the conditioning regimens, the timing of DLI after allo-HSCT, the degree of HLA mismatch, the T-cell dose and subsets and the level of chimerism at time of DLI (Yun et al., 2013). More information on the incidence of GvHD will be provided through the confirmatory trial and the PASS study

Regarding the matched-pair analysis, the relevance of the association between the occurrence of aGvHD upon MM-TK treatment and clinical efficacy in terms of OS, NRM and LFS is limited as the aGvHD patient numbers involved are low (n=29 in the control group and n=13 in the TK-group), the patients that are at risk developing aGvHD upon MM-TK treatment cannot be identified upfront and the effect was not observed in the overall patient comparison. The frequency of GvHD is higher following MM-TK administered as DLI (4/8) as compared to the setting of MM-TK administration with the aim of IR (6/30), probably due to the administration of a higher cell dose/kg than planned in 5 of the 8 patients receiving DLI.

Long-term safety data will also be provided through the PASS conducted and using the EBMT registry where there will be an opportunity to follow-up on the risks of Graft versus Host Disease (GvHD), severe systemic infection CMV and EBV reactivation, febrile neutropenia, hepatic failure, carcinogenicity, genotoxicity, late complications (malignancies, autoimmunity), Immunological events (antibody formation). Information in special populations such as paediatric patients, pregnant women, in patients with renal or hepatic impairment and in the elderly is currently lacking and will be provided through registry data reported regularly (see RMP and Annex II).

Table 22: Effects Table for MM-TK.

Results shown that the overall outcomes at 1–year based on a matched-pair analysis performed 21 days after HSCT.

Effect	Short Description	Unit	Treatment MM-TK	Control historical matched-pair control group from registry	Uncertainties / Strength of evidence	References
Favourable Effects						
OS	Measured from the date of HSCT until death resulting from any cause	% at 1 year	51 (33-69)	34 (25-44)	p=0.007; uncertainties relate to 1) inherent bias related to the limited number of patients (36 MMTK vs 139 controls) , 2) limitations of the indirect nature of the comparison, 3) impact of potential differences in baseline characteristics not included in the matching strategy, 4) long-term efficacy is not known	See this overview
NRM	Defined as any death not preceded by disease relapse/progression*	% at 1 year	20 (8-36)	46 (36-55)	p=0.003; similar uncertainties as described for OS	See this overview
Chronic GvHD	Diagnosed and graded according to standard criteria and computed from the date of HSCT for patients surviving at least 100 days**	% at 1 year	6 (1-19)	23 (15-32)	P=0.02; similar uncertainties as described for OS	See this overview
Unfavourable Effects						
GvHD	Diagnosed and graded according to standard criteria **	% of total number of patients exposed with data available	33% (10 out of 30 MM-TK treated patients from TK007)		GvHD is the only reported AE to be related to MM-TK; can be effectively abrogated upon administration of gancyclovir	See this overview

Effect	Short Description	Unit	Treatment MM-TK	Control historical matched-pair control group from registry	Uncertainties / Strength of evidence	References
Grade 2-4 acute GvHD	Diagnosed and graded according to standard criteria **	% of total number of patients exposed with data available	35%	21%	See GvHD	See this overview
Grade 3-4 acute GvHD	Diagnosed and graded according to standard criteria **	% of total number of patients exposed with data available	8%	9%	See GvHD	See this overview

Abbreviations: OS – overall survival; NRM – non-relapse mortality; GvHD – graft versus host disease; AE – adverse event
Notes:
* Disease relapse/progression was considered as competing even; patients alive without disease relapse/progression were censored at last date known to be alive.
** Death without occurrence of cGvHD was considered competing event.

Benefit-risk balance

Importance of favourable and unfavourable effects

There is an unmet medical need for the MM-TK target population based on the overall grave prognosis due to the underlying haematological disease when disease relapse or progression upon HSCT occurs, with an approximate median overall survival of 6 to 8 months and on the fact that haploidentical HSCT is associated with prolonged immunodeficiency post-transplantation, in particular after extensive treatment for underlying malignancies, the use of T-cell depleted grafts and the use of immunosuppressive drugs. It can take up to about 1–2 years for the complete regeneration of the T-cell and B-cell compartments. During this time, patients are subject to opportunistic infections, which in many cases are fatal. Thus, effective approaches to hasten IR following transplantation, such as MM-TK aims for, are needed to improve outcome and prevent serious, life-threatening complications of HSCT, such as infections.

In this respect, upon transplantation of MM-TK cells, 77% (23 out of 30 patients) of the treated patients in the pivotal study, TK007, achieved IR. When this occurred, it was associated with increased OS, fewer infectious events and reduced NRM. The results from the matched-pair analyses (matched at time of HSCT or at day 21 post HSCT), and the landmark analyses at day 21 post HSCT, are indicative of a considerable benefit of MM-TK treatment in terms of 1 year overall survival and non-relapse mortality. The benefit seen in OS is driven by a reduction in the NRM. The data do not support any claim that MM-TK administration provides protection against relapse of the underlying haematological disease.

Concerning the safety, the pattern of the most common related (S)AEs is not unexpected considering the population and the treatments at hand. Furthermore, none of the deaths have been attributed to

MM-TK treatment. Importantly, the high frequency of death on study (in TK007 and TK008) supports the statement that MM-TK is intended for a life-threatening condition for which there is an unmet medical need.

Furthermore, acute GvHD, being the most commonly reported related AE, was resolved in all cases, and, based on the provided data, activation of the suicide gene by treatment with GCV has contributed to the control of GvHD. It seems that MM-TK is also able to reduce the (chronic) GvHD incidence, which may in part have contributed to the improved NRM, yet the mechanism behind the reduction in cGvHD is not known.

Benefit-risk balance

Despite the remaining uncertainties on the precise magnitude of the benefit, the observed benefits of MM-TK treatment are considered to outweigh the risks associated with the treatment. Therefore the benefit risk of Zalmoxis as adjunctive treatment in haploidentical haematopoietic stem cell transplantation of adult patients with high-risk haematological malignancies – is positive.

Furthermore, it is noted that the data available are not considered sufficiently comprehensive to grant a full marketing authorization however they are of sufficient relevance in the context of a life-threatening disease where an unmet medical need exists. Therefore, a conditional approval is supported subject to submission of comprehensive data within reasonable timelines.

With respect to the application the Applicant argued that Zalmoxis meets the following requirements:

- seriously debilitating diseases or life-threatening diseases;
- orphan medicinal product.

This is endorsed by the CAT.

Based on all the available evidence, a clinical benefit is suggested, with an acceptable safety pattern. Remaining uncertainties can only be resolved by the ongoing confirmatory study. The Applicant has provided sufficient reassurance on the feasibility to complete the ongoing study within a reasonable time frame.

Discussion on the benefit-risk assessment

The CAT is of the opinion that, although comprehensive clinical data referring to the safety and efficacy of the medicinal product have not been supplied, the following requirements for a conditional marketing are met:

- the risk-benefit balance of the medicinal product, as defined in Article 1(28a) of Directive 2001/83/EC, is positive;

The data from this analysis show that the MM-TK treated patients benefited in terms of 1-year OS (40% vs 51%; p=0.03 and 34% vs 51%; p=0.007 for the landmark and matched-pair analysis respectively) and 1-year NRM (42% vs 23% p=0.04 and 46% vs 20%; p=0.003, for the landmark and matched-pair analysis respectively) when compared to the control population. These differences are considered to be of clinical relevance for the target population and taken altogether with an acceptable safety profile, indicate a positive Benefit /Risk balance.

- it is likely that the applicant will be in a position to provide the comprehensive clinical data;

It seems likely that the applicant can provide comprehensive clinical data from the TK008 study, since it is currently ongoing and with the active participation of the EBMT, the number of participating study centres was increased to 40. Considering the increase in the number of participating centers, the completion of the study within a reasonable time frame and the submission of the final study report by

March 2021 seem feasible.

- unmet medical needs will be fulfilled;

There is an unmet medical need for the MM-TK target population based on the overall grave prognosis due to the underlying haematological disease when disease relapse or progression upon HSCT occurs, with an approximate median overall survival of 6 to 8 months and on the fact that haploidentical HSCT is associated with prolonged immunodeficiency post-transplantation, in particular after extensive treatment for underlying malignancies, the use of T-cell depleted grafts and the use of immunosuppressive drugs. It can take up to about 1–2 years for the complete regeneration of the T-cell and B-cell compartments. During this time, patients are subject to opportunistic infections, which in many cases are fatal. Thus, Zalmoxis has shown promising results as an effective approach to hasten IR following transplantation, which is needed to improve outcome and prevent serious, life-threatening complications of HSCT, such as infections.

- the benefit to public health of the immediate availability on the market of the medicinal product concerned outweighs the risk inherent in the fact that additional data are still required

In this context, taking into account the poor prognosis of these patients, the role of Zalmoxis as an adjunctive treatment in haploidentical HSCT is established. Thus on the basis of the above criteria being met, a conditional approval can be supported.

In this respect, as part of a conditional approval, additional data are expected to be obtained from an ongoing confirmatory TK008 trial. In this study DFS was chosen as the primary endpoint; OS, which is a secondary endpoint, is considered to be a more robust outcome measure for showing confirmation of the effects seen in the historical comparison. In this context, based on the clinical and statistical assumptions, the sample size is sufficient to also capture a statistical significant difference in OS. The results from the phase III trial TK008 will be submitted by March 2021.

In conclusion, as the clinical benefit of MM-TK treatment is demonstrated and the safety profile acceptable, a positive benefit-risk of Zalmoxis, in the context of a conditional approval, is considered established. The uncertainties related to the limited data and problems inherent to cross study comparison with historical controls, both in general and more specifically related to MM-TK cannot be solved by additional analyses but require data from the comparative study TK008 in the context of a CMA. Given the current accrual rate and in view of the additional participating study centres, sufficient reassurance on the feasibility of completion of the TK008 trial within an acceptable time frame has been provided.

The CHMP endorse the CAT conclusion on Benefit Risk balance as described above.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Zalmoxis is not similar to Mozobil and Tepadina within the meaning of Article 3 of Commission Regulation (EC) No. 847/200.

Outcome

Based on the draft opinion adopted by the CAT and the review of data on quality, safety and efficacy, the CHMP considers by majority decision that the risk-benefit balance of Zalmoxis as adjunctive treatment in haploidentical haematopoietic stem cell transplantation of adult patients with high-risk haematological malignancies is favourable and therefore recommends granting of the conditional marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

- **Additional risk minimisation measures**

Prior to launch of Zalmoxis in each Member State the Marketing Authorisation Holder (MAH) must agree about the content and format of the educational materials for the Health Care Professionals (HCPs), with the National Competent Authority.

The MAH shall ensure that in each Member State where Zalmoxis is marketed, all HCPs who are expected to prescribe, dispense, and administer Zalmoxis are provided with a guidance document containing the following key elements:

1. Relevant information about the safety concerns of Graft versus Host Disease (GvHD)

During and after treatment with Zalmoxis the physician must be aware of acute and chronic sign and symptoms of GvHD at any time and ensure that either ganciclovir or valganciclovir is available at ward for early treatment of GvHD.

If at any time during or after treatment with Zalmoxis an acute GvHD of grade equal to or greater than 2 or a chronic GvHD develop, the patient has to be treated with ganciclovir at a dose of 10 mg/kg/day divided into 2 administrations intravenously, or valganciclovir 900 mg two times per day orally for 14 days.

In case of GvHD progression after 3 days of treatment with ganciclovir or valganciclovir alone, a standard immunosuppressive therapy has to be added.

Zalmoxis should be administered after a 24-hour discontinuation period of ganciclovir or valganciclovir and immunosuppressive therapy.

2. Relevant information about the safety concern of Concomitant administration of Ganciclovir and Valganciclovir

The treating physician must ensure that patients do not receive ganciclovir or valganciclovir within 24 hours prior to the administration of Zalmoxis. A longer interval might apply in case of renal failure.

3. Relevant information about the safety concern of Concomitant immunosuppressive therapy

Patients should not be administered Zalmoxis in case of:

- Onset of GVHD requiring systemic immunosuppressive therapy
- Ongoing systemic immunosuppressive therapy or administration of granulocyte colony stimulating factor (G-CSF) after haploidentical hematopoietic stem-cell transplantation

Patients could be treated with Zalmoxis 24 hours after the antiviral or immunosuppressive therapy discontinuation.

Zalmoxis shall not be administered to patients with concurrent systemic immunosuppressive therapy as the efficacy of Zalmoxis treatment in early immune reconstitution may be reduced. Immunosuppressive therapy also affects immunocompetent cells as such infused with Zalmoxis. An adequate wash-out period shall be applied prior to infusion of this medicinal product.

4. Remarks on the importance of reporting ADRs and encourage patients to be enrolled into study TK011 (linked with the EBMT registry)

5. A Detailed step-by step description of Zalmoxis administration procedure, also focusing on:

- The room requirements for Zalmoxis administration
- Storage, transport and thawing of Zalmoxis bag
- Surveillance of Zalmoxis efficacy (Immune reconstitution - IR)

To monitor IR, the quantification analyses of CD3+ cells should be performed weekly during the first month after Zalmoxis administration. In absence of IR, an additional Zalmoxis dose has to be administered with an interval of 30 days up to a maximum number of four doses. In case of IR achievement, documented by two consecutive CD3+ cell counts ≥ 100/µl, Zalmoxis treatment has to be stopped.

Obligation to complete post-authorisation measures

Description	Due date
Non Interventional PASS: In order to investigate the safety and effectiveness in real clinical practice as well as long-term safety and effectiveness in all patients treated with Zalmoxis, the MAH should conduct and submit the results of study TK011 using the EBMT registry including all patients treated with Zalmoxis. Progress updates should be submitted yearly with the annual renewal. The clinical study report should be submitted by Q4 2022.	Q4 2022

The CHMP endorse the CAT conclusion on the obligation to conduct post-authorisation measures as described above.

Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14(7) of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

Description	Due date
The MAH shall complete, within the stated timeframe, the below measures: In order to confirm the efficacy and safety of Zalmoxis as an adjunctive treatment in haploidentical haematopoietic stem-cell transplantation of adult patients with high-risk haematological malignancies, the MAH should submit the results of study TK008, a randomized phase III trial of haploidentical HCT with an add back strategy of HSV-Tk donor lymphocytes in patients with high risk acute leukaemia. The clinical study report should be submitted by March 2021. In addition updates on recruitment should be submitted within the PSURs.	March 2021

The CHMP endorse the CAT conclusion on the specific obligation to complete post-authorisation measures for the conditional marketing authorisation as described above.

New Active Substance Status

Based on the CAT review of data on the quality properties of the active substance, the CAT considers that 'Allogeneic T cells genetically modified with a retroviral vector encoding for a truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) and the herpes simplex I virus thymidine kinase (HSV-TK Mut2)' is qualified as a new active substance.

The CHMP endorse the CAT conclusion on the new active substance status claim.

APPENDIX

DIVERGENT POSITIONS

Divergent positions

The undersigned members of the CHMP did not agree with the CHMP's positive opinion recommending the granting of the conditional marketing authorisation of Zalmoxis indicated as adjunctive treatment in haploidentical haematopoietic stem-cell transplantation of adult patients with high-risk haematological malignancies.

The reasons for divergent opinion were the following:

Although the comparison with historical controls shows plausible and promising results, significant uncertainties hamper a conclusion on the clinical benefit as there are critical issues that put the reliability of the available data into question. These critical issues affect all the analyses conducted and cannot be compensated by any additional analyses.

The inherent bias related to the limited number of patients treated with Zalmoxis during the clinical development (studies TK007 + TK008: n=45 patients; pair-matched analysis: n=36 patients) as well as the limitations of the indirect nature of the comparison precludes from firmly concluding about the benefit risk balance.

The bias related to the criteria to receive or postpone the MM-TK treatment (early death, graft failure/rejection, prolonged administration of ganciclovir or immunosuppressive therapy) cannot be fully ruled out, even though all conducted analyses apparently showed the same trend.

The impact of potential differences in baseline characteristics not included in the matching strategy (e.g. median year of transplant, stem cell source, conditioning regimen) is unknown. The Applicant has analysed a set of potential baseline characteristics that may have affected the incidence of cGvHD and this analysis did not reveal an alternative explanation for the observed difference in cGvHD incidence other than MM-TK treatment. However, this analysis does not allow ruling out the impact on other variables. These promising results should be confirmed by the ongoing controlled phase III study before this product can be authorized, especially considering that alternative treatments have been developed and are currently available. It is not considered that the observed benefit clearly outweighs the uncertainties. Therefore, it cannot be concluded that the benefit/risk balance of the product is positive.

London, 23 June 2016

Joseph Emmerich (France) _____

Arantxa Sancho-Lopez (Spain) _____

Sol Ruiz (Co-opted member) _____