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TK008: 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 leukemia

Product:	Haploidentical donor T lymphocytes genetically modified to express HSV-Tk gene
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Synopsis of the Protocol

Title	TK008: 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 leukemia
Sponsor	MolMed S.p.A.
Indication	Patients suitable for haploidentical HCT affected by high risk acute leukemia in 1st or subsequent complete remission, or in relapse.
Objectives	Primary: To compare disease-free survival (DFS) in high risk leukemia patients who underwent haploidentical HCT followed by an add back strategy of HSV-Tk donor lymphocytes or standard haploidentical HCT Secondary: a. To compare overall survival (OS) in the two treatment arms b. To compare the cumulative incidence of non-relapse mortality (NRM) c. To compare the chronic graft-versus-host disease (GvHD)-free, relapse-free survival (GRFS) d. To compare the time to T-cell immune reconstitution e. To compare the engraftment rate f. To compare the cumulative incidence of grade II-IV acute GvHD g. To compare the cumulative incidence of chronic GvHD h. To compare time to GvHD resolution and use of agents with immunosuppressive activity i. To compare the cumulative incidence of relapse (CIR) j. To compare incidence and duration of infectious episodes and infectious disease mortality k. To evaluate the acute and long-term toxicity related to the HSV-Tk infusions l. To assess quality of life (QoL) and Medical Care Utilization (MCU) in both arms
Study design	This is a randomized (3:1), 2-arm, open-label, multicenter, multinational, phase III study with a comparison of an add back strategy of HSV-Tk donor lymphocytes versus standard strategy in high risk acute leukemia patients underwent haploidentical HCT. Patients will be randomly assigned to the treatment group through a centralized randomization process using the following stratification factors: status of the disease at the time of transplantation (e.g. first or subsequent complete remission or relapse), ECOG performance status (0 or 1), and country. In the experimental group A, patients will receive infusion of CD34+ cells plus a dose of T cells (approximately $1x10^4$/Kg) followed by infusion of HSV-Tk genetically modified CD3+ cells In the control group B, the physician will choice if the patient will receive either infusion of CD34+ cells plus a dose of T

	cells (approximately $1x10^4$/Kg) or unmanipulated haploidentical bone marrow or peripheral blood transplantation followed by high-dose cyclophosphamide As part of the Conditional Marketing Authorisation (CMA) granted by the European Medicines Agency (EMA) for the medicinal product, the present phase III trial TK008 has been classified as a Category 2 study, and EMA will review the safety data of the study on an every-6-month basis through the Periodic Safety Update Reports (PSURs), and the benefits and risks of the medicinal product on an annual basis for the renewal of the CMA.
Number of patients	170 patients will be enrolled: 127 patients in the arm A (experimental group) and 43 patients in the arm B (control group)
Study population	**Inclusion Criteria:** 1. Age ≥ 18 years 2. Any of the following conditions: a. AML and ALL in 1st complete remission (CR1) b. AML and ALL in 2nd or subsequent CR c. secondary AML in CR d. AML and ALL in 1st or 2nd relapse or primary refractory 3. Family donor with patient-donor number of HLA mismatches ≥ 2 (full haploidentical), or family donors sharing one HLA-haplotype with the patient 4. Stable clinical conditions and life expectancy > 3 months 5. PS ECOG < 2 6. Serum creatinine < 1.5 x ULN 7. Bilirubin < 1.5 x ULN; transaminases < 3 x ULN 8. Left ventricular ejection fraction > 45% 9. QTc interval < 450 ms 10. DLCO > 50% 11. Patients and donors, or independent witnesses, must sign an informed consent indicating that they are aware this is a research study and have been told of its possible benefits and toxic side effects **Exclusion Criteria:** 1. Patients with life-threatening condition or complication other than their basic condition 2. Contraindication to haploidentical HCT as defined by the Investigator 3. Patients with active CNS disease 4. Pregnant or lactation
Study procedures	**<u>ARM A (experimental group):</u>** Haploidentical HCT with the infusion of CD34+ cells plus a dose of T cells (approximately 1 x 10^4/Kg), followed by: • Infusion of approximately 1±0.2 x 10^7 HSV-Tk genetically modified CD3+ cells/Kg between day

	+21 and day +49 after haploidentical HCT in the absence of spontaneous immune reconstitution (IR has to be documented by two consecutive findings of circulating $CD3^+$ cells ≥ 100/µl) and/or development of GvHD. In absence of immune reconstitution and GvHD further infusions will be administered with the following dosages and timelines: • 30 days (±2 days) after 1st infusion: in the absence of both active GvHD and immune reconstitution (IR has to be documented by two consecutive findings of circulating $CD3^+$ cells ≥ 100/µl) genetically modified lymphocytes will be infused at a dose of $1\pm0.2 \times 10^7$ cells/kg. • 30 days (±2 days) after 2nd infusion: in the absence of both active GvHD and immune reconstitution (IR has to be documented by two consecutive findings of circulating $CD3^+$ cells ≥ 100/µl) genetically modified lymphocytes will be infused at a dose of $1\pm0.2 \times 10^7$ cells/kg. • 30 days (±2 days) after 3rd infusion: in the absence of both active GvHD and immune reconstitution (IR has to be documented by two consecutive findings of circulating $CD3^+$ cells ≥ 100/µl) genetically modified lymphocytes will be infused at a dose of $1\pm0.2 \times 10^7$ cells/kg. **HSV-Tk infusion shall not be infused in case of:** 1. Infections requiring administration of ganciclovir or valganciclovir at the time of infusion 2. GvHD requiring systemic immunosuppressive therapy 3. Ongoing systemic immunosuppressive therapy after haploidentical HCT 4. Administration of G-CSF after haploidentical HCT HSV- Tk cells can be administered after and adequate wash out period (24 hours). **ARM B (control group) :** Haploidentical HCT with either infusion of CD34+ cells plus a dose of T cells (approximately $1x10^4$/Kg) or unmanipulated haploidentical bone marrow or peripheral blood transplantation followed by high-dose cyclophosphamide
Product	Frozen haploidentical donor T lymphocytes genetically modified to express HSV-Tk gene 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. In case of GvHD related to the experimental product: Ganciclovir (10 mg/kg/day in 2 administrations) or Valganciclovir (900 mg bid po) for 14 days

Comparator	Haploidentical HCT with either infusion of CD34+ cells plus a dose of T cells (approximately $1x10^4$/Kg) or unmanipulated haploidentical bone marrow or peripheral blood transplantation followed by high-dose cyclophosphamide
Efficacy	Primary variable: Disease-Free Survival (DFS; Event-Free Survival, EFS) will be measured for all patients from the date of randomization (regardless of disease status at HCT) until the date of relapse (or progression), or death from any cause, whichever occurs first. Secondary variables: a. Overall Survival (OS) will be measured for all patients from the date of randomization until death from any cause. b. Non-relapse mortality (NRM) will be defined for all patients as any death without previous occurrence of a documented relapse (or progression). c. Chronic GvHD-free/relapse-free survival (GRFS) defined as the time from the date of randomization to chronic GvHD, relapse/progression or death from any cause, whichever occurs first. d. Immune reconstitution (IR) will be defined as the time to reach a level of circulating $CD3^+ \geq 100/\mu l$ for two consecutive observations. e. Engraftment will be defined as the persistent blood cells count above a predefined level (ANC $\geq 1x10^9$/L per 3 consecutive days with evidence of donor haematopoiesis; platelets $\geq 50x10^9$/L, unsupported by transfusions, for 7 days) and will be computed from the date (day 0) of transplantation. f. Cumulative incidence of grade 2, 3, or 4 acute GVHD (aGvHD), diagnosed and graded according to standard criteria, will be computed from the transplantation. g. Cumulative incidence of chronic GvHD (cGvHD) diagnosed and graded according to standard NIH consensus criteria, will be computed from the transplantation. h. Duration of GvHD episodes computed from the date of start to the date of resolution and duration of immunosuppressive treatments administered for controlling GvHD i. Cumulative incidence of relapse (CIR) will be defined on the basis of morphologic evidence of leukaemia in bone marrow or other sites. The events are relapses (or progressions). Patients alive without relapse (or progression) will be censored at last contact. Death without experiencing a relapse (or progression) will be considered as competing event
Safety and tolerability	• Adverse Events (AE) and laboratory abnormalities graded according to the CTCAE v 4.02.

	• Serious Adverse Events (SAE) • Suspected Unexpected Serious Adverse Reaction (SUSAR) • Long term follow-up
Pharmacokinetics	Not applicable
Pharmacodynamics	Not applicable
Quality of life/Pharmacoeconomics	• An assessment of the quality of life by questionnaire will be performed before HCT, at month 9 and 12 and then yearly after HCT. The objective will be to summarize and evaluate treatment group differences in patient convenience and satisfaction. • An exploratory pharmacoeconomic analysis will be performed on medical care utilization (MCU). The objective will be to summarize and evaluate treatment group differences in total resource use, and more specifically, in resource use associated with diagnosis, monitoring and treatment of relevant events (i.e., acute and chronic GvHD, infectious episodes, etc). Pharmacoeconomic data may be combined with other data such as cost data or other clinical parameters in the production of a final pharmacoeconomic report. The analysis and reporting of resource use data will be handled separately from the clinical study report
Statistical design	This is a randomized (3:1 allocation experimental arm: control arm), 2-arm, open-label, multicenter, multinational, phase III study. The primary endpoint of the study is disease-free survival (DFS). The disease-free survival following standard haploidentical HCT (with either infusion of CD34+ cells plus a dose of T cells (approximately $1x10^4$/Kg) or unmanipulated bone marrow transplantation followed by high-dose cyclophosphamide) reported in the available studies averages at approximately 30%, and this figure can be used as a reasonable estimate of DFS expected in the control group of the present study. The estimated DFS in the phase II study (TK007) evaluating the add back of HSV-Tk donor lymphocytes strategy was 52%. In order to detect with $\alpha = 0.05$ (2-sided) and $\beta = 0.20$ an increase from 30% to 52% in DFS associated with the experimental treatment (hazard ratio = 0.55), 96 events (relapses or deaths from any cause) need to be observed in the 2 groups combined. To this aim, 170 patients (127 in experimental arm and 43 in control arm) need to be enrolled and followed for at least one year.
Duration of the study	Study duration: 4 years. Recruitment period: 3 years Follow-up for last patient included: 1 year

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1 Introduction

1.1 Background

Many patients with high risk acute leukaemia potentially curable by transplantation of haematopoietic cells (HCT) are generally not considered for such treatment because an HLA identical family donor is lacking.

For these patients, the only curative option is represented by a transplant obtained from a partially HLA matched related donor or haploidentical donor (haploidentical HCT). Such transplants are feasible although still presenting some drawback. When the allograft is not T cell depleted, graft versus host disease (GvHD) becomes the most significant limitation with severe onset occurring in about half of the patients. Therefore, T cell depletion is usually performed to reduce the risk of GvHD but although this is a successful strategy for GvHD prophylaxis, often results in an increased risk of graft rejection and delayed immune reconstitution, thus leading to relapse and post-transplant infections.

The feasibility of haploidentical HCT has increased significantly over the last decade due to several improvements in the conditioning regimen and graft manipulation. The use of high doses of purified CD34+ haematopoietic progenitor cells combined with a highly immunosuppressive chemo-radiotherapy treatment has provided sustained engraftment in over 90 percent of transplant recipients in spite of extensive T cell depletion. The incidence of acute and chronic GvHD has been reduced due to the improvements in T cell depleting technology and the use of pre-transplant anti thymocyte globulin (ATG) [1]. In this setting the major causes of morbidity and mortality still relate to delayed immune reconstitution resulting from the paucity of infused immune cells.

It was hypothesized and confirmed in a series of clinical trials that immune recovery following haploidentical HCT could be improved by the infusion of donor T lymphocytes engineered to express the thymidine kinase of the Herpes Simplex Virus (HSV-Tk), a suicide gene activated upon ganciclovir administration. Recent data related to a multicenter Phase II trial (TK007) identified a dose of transduced cells corresponding to 10^7c/kg, proved to be sufficient for the induction of a stable level of immune reconstitution. These levels were obtained within a median time of 77 days (66-88) from transplant and 21 days (14-28) from the infusion of HSV-Tk cells leading to a reduction in late mortality due to infections[2].

The objective of the proposed trial is to demonstrate the superiority in terms of non-relapsed mortality reduction and overall survival improvement of HSV-Tk add back strategy versus a standard strategy following haploidentical HCT.

1.2 Drug Description

The drug product is defined as frozen haploidentical donor T lymphocytes genetically modified to express the HSV-Tk gene 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. It is a patient specific product prepared starting from a lymphocytoaphaeresis of a dedicated donor.

The retroviral vector SFCMM-3 Mut2 is generated from SFCMM-3 vector (vector used for a phase I-II TK007 trial) by site-directed mutagenesis with the introduction of a silent T→C transition at the splicing donor site.

The mutation introduced in the SFCMM-3 Mut2 vector has been proved to avoid the generation of the HSV-Tk gene spliced form thus improving the safety of the drug substance. Therefore, SFCMM-

3 Mut2 vector was chosen for the development of a packaging cell bank system and for the production of retroviral vector to be used in the proposed Phase III clinical study.
HSV-Tk gene encodes the thymidine kinase enzyme of Herpes Simplex Virus I.
The derived enzyme is functional and used both in vitro and in vivo to selectively eliminate the transduced cells in presence of ganciclovir.
ΔLNGFR is the gene that encodes for the intracellular truncated form of the low-affinity receptor of Nerve Growth Factor.
The loss of the intracellular domain makes the molecule inert from a functional point of view, while allowing for correct cell surface expression. Such localization allows for the recognition of cells that express ΔLNGFR using FACS analysis with a specific antibody.
A potential problem associated with the thymidine kinase/ganciclovir system is the emergence of ganciclovir resistance in HSV-Tk transduced cells. This is of particular importance, since the relative proportion of cells which are resistant to ganciclovir may rapidly increase through the course of treatment.
The molecular mechanism responsible for ganciclovir resistance consists in a 227 base pair deletion in the HSV-Tk gene. This deletion has been documented both *in vitro* in human primary T cells transduced with SFCMM-3 vector as well as with a different retroviral vector (G1TkSvNa; and *in vivo* in blood samples from 12 patients treated with HSV-Tk transduced donor T cells[3].
The cause of this deletion is the presence of nucleotide sequences in the HSV-Tk mRNA which act as splice sites to cause the production of a proportion of virus particles having an aberrant form of the gene, the remaining viral particles having the full length gene.
SFCMM-3 Mut2 vector has been generated from the parental SFCMM-3 vector by site-directed mutagenesis, with the introduction of a silent T>C transition at the splice donor site. The presence of this mutation substantially prevents the splicing phenomenon.
The SFCMM-3 Mut2 construct has been used to transfect the ecotropic GP+E86 packaging cells line (ATCC n° CRL-9642). The supernatant from the transient transfection was harvested and used to infect the packaging amphotropic GP+*env* Am12 packaging cell line (ATCC n° CRL 9641) from now on referred to as Am12.
The integration of the construct in the packaging cell lines DNA allows for the production of retroviral vectors with an amphotropic spectrum, able to infect a broad range of mammalian cells, including human cells. The Am12 bulk cell population was selected following transduction on the basis of expression of ΔLNGFR using specific antibodies conjugated to magnetic beads in order to recover positively transduced cells.
The resulting cell population was than plated in limited dilution conditions in order to obtain single producer cell clones.
The clones were tested for expression of ΔLNGFR, sensitivity to ganciclovir, good growth capacity in culture, efficiency of transduction of T lymphocytes, stability, and absence of contaminating adventitious viruses and of replication competent forms, as described elsewhere.

1.3 Drug activity

HSV-Tk Mut2 is the gene encoding for a mutated form of the thymidine kinase of Herpes Simplex Virus I and once integrated into donor lymphocytes DNA, makes them sensitive to Ganciclovir (GCV). Following its administration, GCV is firstly monophosphorylated by HSV thymidine kinase enzyme expressed in the genetically modified cells and then transformed to the triphosphate form by other cellular kinases.
This active, triphosphopate form of ganciclovir, induces the blockage of synthesis of newly DNA strand during mitosis, and in this way provokes the death of proliferating cells.

The gene ΔLNGFR encodes for a low affinity form of the nerve growth factor (NGF) receptor, which has been deleted of its intracellular portion in order to be no longer able to transmit signals.
The expression of ΔLNGFR on the cell membrane allows for the immunoselection of genetically modified cells using monoclonal antibodies conjugated to magnetic beads. Furthermore, the expression of the ΔLNGFR protein is used *in vivo* as a marker of genetically modified cells once infused into the patients and allows the assessment of the Drug Product persistence in the human body following treatment, possible cell population expansion or reduction and characterization, in terms of lymphocyte subtype and state of activation.

1.3.1 Non clinical studies

1.3.1.1 In vitro tolerability studies

The ectopically expression of transgenes in genetically modified lymphocytes does not seem to alter either their proliferative potential or functionality.
In particular, repeated laboratory tests on genetically modified lymphocytes have shown:
The expression of these proteins does not affect the phenotype of the cells, which continue to express the characteristic lineage, activation and adhesion molecules. The transduced and cultivated lymphocytes express the panlymphocyte marker CD2. Even though, a large percent of the transduced lymphocytes are T lymphocytes (CD3+), a variable number of cells (between 3 and 18%) have a NK phenotype (CD2+, CD3-, CD56+). NK cells are one of the subgroups implicated in the antitumoral response. CD3+ lymphocytes after manipulation, generally retained an inversion in the CD4/CD8 ratio in favour of the cytotoxic population which represent the effector arm of anti-tumoral and anti-viral immune responses. The *in vitro* stimulation required for transduction results in a polyclonal activation of the cells. With the aim of characterizing the level of activation resulting from *in vitro* manipulation, the expression of the following activation markers was analyzed, on the manipulated cells 14 days after stimulation: CD25, HLA-DR, CD69, CD95, CD45RA, CD45RO and CD28. Essentially, all the transduced lymphocytes showed to have encountered antigen (CD45RA-, CD45RO+) and the proportion of cells displaying activation markers was variable: 14 days after stimulation a large part of the transduced cells expressed HLA-DR and CD95 but have lost membrane expression of CD25 and, in some cases, CD69 suggesting that the cells have reached a new stage of quiescence. Furthermore, it has been noted that a significant percentage of the transduced cells maintained expression of CD62L, a molecule crucial for the migration of lymphocytes into lymph nodes, suggesting that the *in vitro* manipulation does not alter the homing capacity of T lymphocytes. An elevated percentage of manipulated lymphocytes maintained expression of CD28. Physiologically, the counter of CD28 with its ligand results in the recruitment into proximity with the TCR of molecules able to reduce the threshold for activation of the lymphocytes. Therefore, CD28+ transduced T lymphocytes should be rapidly activated with low doses of antigen in comparison with unmanipulated lymphocytes[4]. All the phenotypic alterations observed in transduced cells were also noted on non-transduced cells that were activated and cultured under the same conditions. This implies that the *in vitro* activation/culture, and not the selection/transduction, is responsible for the observed changes.
No notable changes were observed in the transduced cells in terms of survival and proliferation evaluated as spontaneous annexin 5 expression or tritiated thymidine incorporation. These data suggests that the introduction of new genetic material did not contribute to cell immortalization, transformation, or any other cellular toxicity[5].
Engineered cells maintain the same IL-2 dependency as unmanipulated cells in terms of survival and growth. When deprived of IL-2, the culture growth slows and induces programmed cell death.

The expression HSV-Tk, that renders the lymphocytes sensitive to ganciclovir, but in its absence of any alterations in terms of proliferation, lytic activity and cytokine production in the transduced cells could be observed. Indeed, lymphocytes genetically modified with SFCMM-3 maintain the same proliferative and cytotoxic activities then untransduced T cells as assessed in a ^{51}Cr release assay performed after 7 days stimulation in the presence of the below listed targets relevant in the context of allogeneic transplantation:

- allogeneic lymphocytes;
- autologous cell lines that have been immortalized with Epstein-Barr;
- autologous cell lines pulsed with the M58-64 peptide from influenza virus;
- autologous fibroblasts infected with cytomegalovirus.

Furthermore, stimulation of both unmanipulated cells or genetically modified lymphocytes with autologous fibroblasts infected with cytomegalovirus showed that the same antigen specificity was obtained, thus indicating that the manufacturing process does not impair functionality of lymphocytes against immunodominant cytomegalovirus antigens.

1.3.1.2 In vivo efficacy and tolerability

The efficacy and tolerability of the HSV-Tk/GCV suicide system has been shown in several animal models. There have been used:

- The murine T lymphoma YC8 that when inoculated into balb/c mice gives rise to solid tumors (after subcutaneous injection, sc) and metastasis (after intravenous injection, iv). 10^4 YC8 cells transduced and expressing HSV-Tk were infused both sc and iv into 2 groups of 10 balb/c mice per group. 5 days after infusion 5 mice/group received a dose of ganciclovir of 100 mg/kg/day ip for 5 days. The administration of ganciclovir significantly reduced the dimensions of the solid tumors and both the number and dimension of metastasis (data available at H.S. Raffaele Scientific Institute);
- Hagenbeek, Department of Hematology, Utrecht, has developed a model of GvHD in the Brown Norway Rat (BN). In this model, splenic T cells from Wag/Rij rats (donor) are transduced with the vector SFCMM-3 and then infused together with hematopoietic stem cells in to the recipient BN after a conditioning regime using TBI + ATG + FK506. The result is an acute GvHD. The administration of ganciclovir at the development of GvHD postpones the death due to GvHD from day 18-20 to day 28-30. Ganciclovir given in prophylaxis significantly augments the survival;
- J. Apperley, Department of Hematology, Hammersmith H., London, has developed a murine model of transduction of RMA cells with SFCMM-3. RMA transduced with SFCMM-3 are inoculated sc at variable doses from 105-106 in C57BL/6 mice. One cohort of mice was then treated with ganciclovir at 20 mg/kg ip in prophylaxis the day of inoculation of the cells, while the second cohort was treated the moment at which the tumor nodule reached 1 cm in diameter. The administration of ganciclovir prevents (in the first cohort) or reduces (in the second cohort) the development of tumors[6];
- P. Tiberghein, Centre Hospitalier Universitoire (CHU), Besancon, has developed a model in balb/c and C57/BL/6 mice transplanted with FBV stem cells with an addition of splenocytes from HSV-Tk+ mice (derived from the FBV mouse which is transgenic for HSV-Tk). At emergence of the GvHD, ganciclovir augments the survival to 40-60% from 0-6% in the control mice[7];

- M. Helene has developed a murine model consisting of stem cell administration B10.A + 5x106 B10.A (5R) splenocytes which are transgenic for HSV-Tk into DBA/2 mice that have been lethally irradiated. In this model, the prophylactic administration of ganciclovir in the third and tenth days reduces the recipient mouse GvHD induced death from 50% to 0%[8];
- Bondanza et al.[9] developed a GvHD model using NOD/SCID mice treated with human SFCMM-3 transduced and selected lymphocytes. In this setting, GvHD induced by TK+ lymphocytes could be controlled by GCV administration.

1.3.2 Clinical studies

A phase I/II clinical trial, named TK007, was completed in its main cohort which consisting in the administration of HSV-Tk lymphocytes (genetically modified with SFCMM-3 #35 vector) as fresh product after haploidentical HCT[10]. The recruitment of additional patients for a sub-cohort receiving HSV-Tk lymphocytes frozen at the end of the transduction procedure was pursued.
The aim of the TK007 study was to obtain immune reconstitution as well as reduction of infective episodes and disease relapse in patients with haematological malignancies who underwent haploidentical HCT (and subsequent T lymphocytes infusions) selectively controlling GvHD.
According to the protocol a patient was considered evaluable for the main end point of the study (immune reconstitution) if he/she received at least one dose of HSV TK transduced T cells.
In presence of acute Graft versus Host Disease related to the investigational product, ganciclovir was administered in order to eliminate of the HSV TK transduced T cells and to the resolve of the adverse reaction. The clinical protocol foresaw infusion of escalating doses of HSV-Tk cells administered immediately after the transduction process. The escalating dose were the following:

- 1st infusion: 1 x 10^7 CD3+ c/kg or alternatively 1x10^6 CD3+ c/kg;
- 2nd infusion: 1 x 10^7 CD3+ c/kg;
- 3rd infusion: 1 x 10^6 CD3+ c/kg + IL-2 (6.000.000 IU/m2 sc x 5 days);
- 4th infusion: 1 x 10^7 CD3+ c/kg + IL-2 (6.000.000 IU/m2 sc x 5 days).

57 patients were enrolled and five did not undergo HCT. Among 52 transplanted patients (median age, 49 years; 22 males and 30 females) 30 patients (17 in the fresh cohort and 13 in the frozen cohort) were treated.
22 patients did not receive treatment because of early death (n=12), graft failure/rejection (n=7), prolonged administration of ganciclovir or immunosuppressive therapy (n=3). The following diagnoses were reported: 26 AML, 10 sAML, 3 ALL, 7 RAEB-T/MDS, 1 biphenotipic AL, 1 CML-AP, 4 NHL/HD.
Status of disease at date of haploidentical-HCT: 21 patients in relapse/progression/advanced disease, 31 patients in CR.
23 patients (77%) out of 30 treated achieved immune reconstitution (IR), with a median time of 77 days from haplo-HCT and 31 days from first HSV-Tk infusion.
No difference between frozen and fresh cohort were observed when comparing time to IR in the two cohorts. These results denote a substantial bio-similarity of frozen cells compared to the fresh cells.
The HSV-Tk add back strategy provide the basis for a rapid and efficient reconstitution, with absence of severe toxicity. Safety data referred to 30 treated patients; the adverse events (AEs) related to the experimental product are reported in table 1.

Table 1. **TK007: adverse events related to HSV-Tk by 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 graft versus host disease	**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 graft versus host disease	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
Post-transplant lymphoproliferative disorder	0	0.0	0	0.0	1*	3.3	0	0.0	0	0.0	1	3.3

NA: not applicable *Same patient TK8

The most frequently HSV-Tk related AEs, reported in at least 10% of patients, was represented by GvHD (in bold type in table 1). No grade 5 related-events were reported.
The potential risk of development of GvHD could be efficiently controlled by the suicide system operated by ganciclovir/valganciclovir thus rendering the therapy safely administrable. In the current trial, acute GvHD was reported for 10 patients out of the 30 treated patients.
The most common adverse event related to HSV-Tk cells was represented by graft-versus-host disease (GvHD). Among 30 treated patients, acute graft-versus-host disease (GvHD) occurred in 10 patients (33%) with a median time to onset of 90 days (range, 20 to 162) after HCT and 32 days (range, 8 to 91) after the last infusion of HSV-Tk cells.
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 HCT and last infusion, respectively, and fully resolved after 107 days.
By timing of onset, acute GvHD developed in 6 of 30 patients (20%) who initially received HSV-Tk cells during the treatment phase at a median of 94 days from HSTC, 48 days from first infusion of HSV-Tk cells and 17 days from immune reconstitution.
Additionally, acute GvHD occurred in 4 of 8 patients (50%) who had received subsequent infusions of HSV-Tk cells given as donor lymphocyte infusion (DLI) for treating disease relapse or progression at a median time of 21 days after last HCT and 32 days after first infusion of HSV-Tk cells. None of these four patients had previously experienced HSV-Tk-related GvHD and none of other four retreated patients developed de novo GvHD.
The clinical treatment and outcome of patients experiencing GvHD was as follow:

- 1 patient grade 1 (skin), no treatment;
- 7 patients grade 2 (skin), 7 treated with GCV and 5 also with steroids;
- 1 patient grade 3 (skin), treated with GCV alone;
- 1 patient grade 4 (gut and liver), treated with GCV, steroids and mycophenolate;

- 1 patient chronic GvHD (skin, mouth and eyes), treated with GCV and mycophenolate mofetil.

GvHD completely resolved in all cases. No GvHD related deaths occurred in this study.
Despite the small number of patients, our results indicate no differences in the efficacy and safety of GCV and valGCV for the treatment of GvHD in patients treated with HSV-Tk cells, as already demonstrated for the treatment of CMV infection.
In addition, RCR detection was performed on all TK007 treated patients pre-post infusion, at 3 months, 6 months, at one year and then yearly post-treatment, in compliance with the FDA guideline (FDA/CBER, *"Guidance for Industry, Supplemental Guidance on testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of patients in Clinical Trials Using Retroviral Vectors"*, October 2000). The outcome of all performed RCR tests was negative.

1.4 Rationale

AML is currently classified combining clinical and laboratory data in three broad prognostic groups: favourable, standard (or intermediate), and unfavourable. The favourable prognostic subgroup, which includes approximately 20 percent of cases among patients who are 60 years of age or younger, is defined by the presence of leukemic blasts with the t(15; 17), t(8;21), or inv(16) mutation or molecular evidence of these abnormalities. These mutations are more frequent in younger patients who have high (more than 85 percent) rates of complete remission and a relatively low risk of relapse (30 to 40 percent).
On the other end of the spectrum there is the unfavourable prognostic subgroup, which includes approximately 15 percent of the cases among patients who are 15 to 60 years of age.
These unfavourable cases are defined by the presence of leukemic blasts with cytogenetic abnormalities involving more than two chromosomes, monosomies of chromosome 5 or 7, deletion of the long arm of 5 (del5q), or abnormalities of the long arm of chromosome 3. These abnormalities are more frequent in older patients and in patients with secondary AML, but even among younger patients, the survival rate is less than 20 percent at five years. Between these two groups there are patients who are characterized as having a standard (or intermediate) risk of relapse. The leukemic blasts of these patients have either a normal karyotype or cytogenetic abnormalities that are not included in the definition of the other subgroups. In some series this includes patients with cytogenetic abnormalities of 11q23, whereas in others these patients are included in the unfavourable prognostic subgroup. Patients older than 60 years generally have a poor prognosis, with a probability of survival at five years of less than 10 percent. In summary, there is general consensus in considering high risk AML with the following conditions at diagnosis: secondary AML following Myelodysplastic Syndrome; WBC > 50 x 10^9 L; marrow blasts > 15% after course 1 of induction chemotherapy; FAB subtypes M0,5,6,7; high risk cytogenetic group: -5/del5q, del(7q), 3q-, complex, t(6;9), t(9;22), 11q, 21q, 17p; standard risk cytogenetic group (normal, +8) with FLT3 internal tandem duplication, or hepatosplenomegaly, extramedullary disease at diagnosis. Although the outcome of patients with AML has improved because of cytarabine and anthracycline based chemotherapy and introduction of HCT, relapse continues to represent the major cause of death in the vast majority of patients. The probability of relapse depends on risk factors such as age, pre-treatment, cytogenetics and number of cycles of induction chemotherapy required for attaining the first complete hematologic response (CR). In addition, treatment of AML in first relapse is associated with relatively low response rates. Whenever second CR is attained, the median duration of the second relapse-free interval (RFI) is generally considerably shorter than that of the first RFI. Factors predicting outcome of patients with AML in first relapse have been reported, and include RFI, age, and cytogenetics. However, proposed

stratification methods for selecting therapies for patients with relapsed AML have only been based on the duration of RFI, thus neglecting the influence of other known prognostic factors. Predictive scores for patients with AML in first relapse that include more covariates could be generally applicable, if they would furnish a simple and statistically valid prognostic index. Recently, HOVON cooperative group proposed a score including RFI, cytogenetics, age and previous HCT as covariates[11]. According to this large retrospective analysis, allogeneic HCT is indicated in vast majority of patients with AML in CR2. The prospect of transplantation would increase if it is intended to deliver earlier as soon a CR2 obtained. Similarly to AML, ALL is currently classified combining clinical and laboratory data[25-33]. According to standard consensus criteria age is one of the most important prognostic factor. OS continuously decreases with increasing age from 34-57% below 30 years to 15-17% above 50 years. An elevated WBC at diagnosis is associated with a higher relapse risk, with risk of complications during induction and with an increased risk of CNS relapse. A complete immunologic characterization at diagnosis is required to identify subtypes with different presentations and prognoses. Pro-B-ALL and/or $t(4;11)^+$ ALL is considered a poor prognostic subgroup, it appears to be particularly susceptible to SCT. The survival was 74% for all of sibling SCT in CR1, indicating that pro-B-ALL has the most favourable outcome after SCT compared to other ALL subgroup in the GMALL studies. Common/pre-B-ALL bears a large proportion of Ph/BCR-ABL^+ ALL condition that identified a population at very high risk, with absolute indication to allogeneic transplantation. Many group have confirmed the superior outcome of T-lineage ALL compared to B-lineage. The LFS was significant poorer for early T-ALL (25%) and mature T-ALL (28%) compared to thymic T-ALL (63%). The former subgroup are considered as indications for SCT in CR1 in the GMALL studies. Besides age the most relevant prognostic factor in ALL is the achievement of CR, further prognostic factor are delayed time to CR or response to prednisone therapy. A more accurate approach to assess individual response is minimal residual disease (MRD), actually under evaluation for broader application. According to the literature it is possible identified adverse prognostic factors as: WBC >30.000 in B-ALL, >100.000 in T-ALL, complex karyotype [t(9;22)/BCR-ABL, t(4;11)/ALL1-AF4, t(1;19)/PBX1-E2A], age >35 years, late CR, MRD persistence $>10^{-4}$ for 3-4 months. According to EBMT standard practice recommendations patients with ALL with poor prognostic features, or delayed time to obtain remission are candidates for allogeneic HCT in first remission. Allogeneic HCT for standard risk patients in first CR should be performed within a clinical protocol. Patients who relapse after chemotherapy and achieve a second CR are candidates for allogeneic HCT, in fact outcome of HCT is superior to chemotherapy alone. A steady stream of advances in allogeneic HCT has not only improved the clinical outcome in a variety of malignant and non-malignant diseases but also widened the indications for such transplants[12,13]. The immunological role of donor T lymphocytes in the eradication of the tumours is universally recognized and the added Graft versus Leukaemia effect (GvL) makes this therapeutic method the preferential choice for patients who have a consanguineous or marrow bank donor. Furthermore donor lymphocytes infusions (DLI) have been shown to be effective in the treatment of a large number of post-transplant relapses, with a success rate of between 15 to 80 % depending on the type of disease. The infusion of donor T lymphocytes also mediates the reconstitution of the immune responses against viruses (for example induced Epstein Barr Virus lymphoma or Cytomegalovirus reactivation) and fungi, as demonstrated by the lower incidence and severity of these infections in the context of unmanipulated transplantation versus T depleted transplant. However, the infusion of donor T lymphocytes increases the risk of GvHD and the incidence and severity of the disease correlates with the number of infused lymphocytes. Currently, the therapeutic options for GvHD are limited to immunosuppressive agents and can result in severe infections and/or disease relapse. In recent years, different strategies for the prevention and treatment of GvHD have been developed. These include the modulation of the infusion parameters (time and number of administrations and

dose of infused lymphocytes), enrichment of lymphocytes thought to be responsible for the antitumoral (GvL) or antiviral activities and depletion of the population responsible for GvHD. Although widely recognized as effective therapeutic tool, the sources of hematopoietic stem cells from HLA-identical siblings, are available to only about 30 percent of potential recipients. For situations in which a matched unrelated donor cannot be found within a reasonable time, haploidentical family donors has emerged as an attractive source of stem cells[14]. The EBMT Acute Leukaemia Working Party collected data on 273 haploidentical transplants from 75 centers in adults patients with de novo acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL) reported to the EBMT from 1995 to 2002 and analysed the outcome of this procedure according to the known risk factors. Overall, 170 AML patients, median age 38 years (16-70) underwent transplantation in CR1 (39), CR2 (34) or in advanced disease (97). In addition 103 ALL patients, median age 26y (16-56). Underwent transplantation in CR1 (31), CR2 (22) or in advanced disease (50). Primary engraftment was documented in 87% AML and in 83% of ALL patients. The cumulative incidence of acute GvHD >=II was 16% in AML and 15% in ALL. At a median follow-up of 19 months (1-85), the estimated leukaemia-free survival (LFS) at 2 years was 39% for AML and 28% for ALL transplanted in CR1. The non-relapse mortality (NRM) at 2 years in AML was 52% for CR1 and 44% for ALL in CR1. Analysis on center effect on LFS, RI and NRM showed no differences on outcome. In conclusion, the EBMT registry analysis of allogeneic transplantation from haploidentical family donors confirmed relevant LFS at 2 years for patients with high risk AML while revealing a pattern of late Transplant related Mortality and late relapse due to failure of immune reconstitution[15]. However, although feasible, one of the main problems of haploidentical HCT is represented by the delayed immune reconstitution[1]. The risk of severe infections remains high for several months and CD4+ cells reconstitution could take more than 10 months. The low number of lymphocytes infused with the graft, the degree of HLA disparity, a reduced thymic function in adults and differences in host/donor antigen presenting cells are all contributing causes. Attempts to overcome this problem with infusions of donor lymphocytes have been associated with a high incidence of GvHD [16-18].The infusion of HSV-Tk transduced lymphocytes should allow for early immunological reconstitution and reduce the risk of infections and disease relapse. Data in the literature showed that the infusion of lymphocytes transduced with HSV-Tk were able to control in principle the development of GvHD without resulting in any severe side effect related to the in vitro manipulation of lymphocytes[2,19-22]. A completed phase I/II multicenter trial (TK007) was designed based on historical data demonstrating the anti-leukemic potential of HSV-Tk cells in HLA-matched setting and their ability to control GvHD. In TK007 study, among the 30 treated and the 23 immune-reconstituted patients the non-relapse mortality was 30% and 17%, the disease-free survival was 30% and 39% and the overall survival was 40% and 52%, respectively.

It has been shown in animal models that both graft rejection and GvHD after histoincompatible bone marrow transplantation (BMT) can be inhibited by post-transplant administration of high-dose cyclophosphamide, which is known to be highly toxic to lymphocytes proliferating in response to recent antigen stimulation. Based on this experience, Luznik et al[41] first demonstrated in 68 patients with hematologic malignancies the feasibility of unmanipulated T-cell repleted BMT from haploidentical related donors followed by post-transplant high-dose cyclophosphamide on days 3 and 4. As GvHD prophylaxis, the patients received also tacrolimus and mycophenolate mofetil. Low incidences of acute GvHD (grades 2-4, 34% and grades 3-4, 6%) and non-relapse mortality at 1 year (15%) were observed, although there was a relatively high incidence of graft rejection (13%) and relapse rate at 1 year (51%). After a median follow-up of 745 days, the actuarial disease-free survival (DFS) at 1 year was approximately 30%.

The results of multicenter, prospective phase II TK007 trial provide the basis for the present phase III trial aimed to compare the add back HSV-Tk strategy versus a standard haploidentical HCT, which consists of haploidentical HCT either with infusion of CD34+ cells plus a dose of T cells (approximately 1 x 10^4/Kg) or with unmanipulated haploidentical BMT, at Investigator discretion.

2 Objectives

The objective of this randomized trial is to demonstrate the efficacy in increasing disease-free survival (DFS) of HSV-Tk add back strategy versus a standard strategy, following haploidentical HCT in patients with acute high risk leukaemia.

2.1 Primary aim

To compare disease-free survival (DFS) in high risk leukaemia patients who underwent haploidentical HCT followed by add back strategy of HSV-Tk donor lymphocytes or standard haploidentical HCT.

2.2 Secondary aims

a. To compare overall survival (OS) in the two treatment arms
b. To compare cumulative incidence of non-relapse mortality (NRM)
c. To compare the chronic graft-versus-host disease (GvHD)-free, relapse-free survival (GRFS)
d. To compare time to T-cell immune reconstitution
e. To compare engraftment rate
f. To compare cumulative incidence of grade II-IV acute GvHD
g. To compare cumulative incidence of chronic GvHD
h. To compare time to GvHD resolution and use of agents with immunosuppressive activity
i. To compare cumulative incidence of relapse (CIR)
j. To compare incidence and duration of infectious episodes and infectious disease mortality
k. To evaluate the acute and long-term toxicity related to the HSV-Tk infusions
l. To assess quality of life (QoL) and Medical Care Utilization (MCU) in both arms

3 Trial design

Prospective, randomized (3:1), 2-arm, open label, multicenter, multinational phase III trial.
As part of the Conditional Marketing Authorization (CMA) granted in August 2016 by the European Medicines Agency (EMA) for the medicinal product (www.ema.europa.eu/EPAR/Zalmoxis), the present phase III trial TK008 has been classified as a Category 2 study, and EMA will review the safety data of the study on an every-6-month basis through the Periodic Safety Update Reports (PSURs), and the benefits and risks of the medicinal product on an annual basis for the renewal of the CMA.

4 Patient selection criteria

4.1 Target population

Patients suitable for haploidentical HCT affected by high risk acute leukaemia in 1st or subsequent complete remission or in relapse.

4.2 Inclusion criteria

1. Patients ≥ 18 years
2. Any of the following conditions:
   a. AML and ALL in 1st complete remission (CR)
   b. AML and ALL in 2nd or subsequent CR
   c. Secondary AML in CR
   d. AML and ALL in 1st or 2nd relapse or primary refractory
3. Family donor with patient-donor number of HLA mismatches ≥ 2 (full haploidentical) or family donors sharing one HLA-haplotype with the patient
4. Stable clinical conditions and life expectancy > 3 months
5. PS ECOG < 2
6. Serum creatinine < 1.5 x ULN
7. Bilirubin < 1.5 x ULN; transaminases < 3 x ULN
8. Left ventricular ejection fraction > 45%
9. QTc interval < 450 ms
10. DLCO > 50%
11. Patients and donors, or independent witnesses must sign an informed consent indicating that they are aware this is a research study and have been told of its possible benefits and toxic side effects.

4.3 Exclusion criteria

1. Patients with life-threatening condition or complication other than their basic condition
2. Contraindication to haploidentical HCT as defined by the Investigator
3. Patients with active CNS disease
4. Pregnant or lactation. Patients both males and females with reproductive potential (i.e. menopausal for less than 1 year and not surgically sterilized) must practice effective contraceptive measures throughout the study. Women of childbearing potential must provide a negative pregnancy test (serum or urine) within 14 days prior to registration.

4.4 Conditioning regimens

Investigators can use conditioning regimens for haploidentical HCT according to Institutional clinical practice.
Some suggested conditioning regimens have been listed in appendix A.

4.5 Recommended Supportive Care

Investigators can use supportive care (including prophylaxis and treatment with antibacterial, antiviral, antifungal and antimycobacteria therapy), according to Institutional clinical practice.

5 Experimental arm (Arm A)

5.1 Drug information

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. It is a patient specific product prepared starting from a lymphocytoaphaeresis of a dedicated donor.
The lymphocytoaphaeresis is performed at the clinical center according to common clinical procedures and sent to MolMed for the transduction process.
For dose preparation, the frozen peripheral blood mononuclear cells (PBMC), isolated from lymphocytoapheresis by density gradient, are thawed, stimulated, transduced in the presence of the SFCMM-3 Mut2 #48 retroviral vector and positively transduced cells immunoselected using an anti-LNGFR specific monoclonal antibody (mAb 20.4) conjugated with magnetic microbeads, in order to obtain a highly purified population of positively transduced cells.
After the culture for expansion, the transduced lymphocytes are harvested and re-suspended in freezing medium (Saline solution + HSA 7% + 10% DMSO) at the concentration of 5-20x10^6 cells/ml in ethylene vinyl acetate EVA CryoMACS freezing bags, frozen in decreasing temperature controlled conditions and stored in liquid nitrogen vapour phase.

5.2 Drug Product supply

The genetically modified T donor lymphocytes will be supplied by MolMed S.p.A. as one individual treatment dose in a 50-500 mL ethylene-vinyl-acetate cryo bags. Further details about procedures and forms are provided in the Study Manual.
The investigator or designee is responsible for investigational product accountability.
To this end, it is assumed that all clinical trial supplies will be delivered to and under the responsibility of a suitably qualified and authorized person, who will document drug disposition and accountability for the duration of the trial.

5.3 Drug Product formulation, packaging, labelling and storage

The genetically modified T donor lymphocytes will be supplied in EVA cryo-bag, in saline solution containing 7% HSA and 10% DMSO at the concentration of 5-20x10^6 cells/ml, in a volume of 10-100 ml
The primary packaging of the drug product will bear a label with at least the following information: the protocol name, the Principal Investigator name, the batch number, the patient code, the donor code, the storage conditions, the manufacturing date, the total number of cells, the route of administration, the Sponsor name, “For clinical trial use only” and the expiry date. Text might be in different order on the labels and will be adapted to the country specific local requirements.
The drug product will be stored in liquid nitrogen vapor phase in a locked facility.
The donor lymphocytoaphaeresis, the genetically modified T donor lymphocytes and all documentation related to storage, manipulation, testing, production and shipping will be recorded and traceability will be guarantee by dedicated logbooks.

5.4 Drug administration

Before the infusion, the cryo-bag with the transduced donor T-lymphocytes, must be thawed, when completely thawed the entire volume of the bag must be infused as quickly as possible. The recommended infusion time is around 20-60 minutes.
In order to administer completely the HSV-Tk cells it is recommended to wash the bag 2-3 times with physiological solution using a sterile solution. The washing procedure must be performed during the infusion in a sterile way. Further details about procedures will be provided in the Study Manual.
After the infusion, the cryo-bag will be disposed of according the local site procedures for the biological material.
Information on shedding and any precautions required are reported on safety data sheet.

During infusion of HSV-Tk cells monitoring of vital signs (pulse rate and blood pressure) should be done:

1. before infusion (baseline)
2. after the completion of infusion:
    a. every 15 minutes for the first hour,
    b. every 30 minutes for the second hour, and
    c. hourly through the end of the fourth hour.

5.5 Dosage schedule

Patients randomized for arm A will receive the treatment, up to a maximum of 4 infusions, with an interval between each infusion of 30 days (±2 days), in the absence of spontaneous immune reconstitution (IR has to be documented by two consecutive findings of circulating $CD3^+$ cells ≥ 100/µl) and/or development of GvHD.
The 1st infusion of HSV-Tk genetically modified T-lymphocytes will be administered between day +21 and day +49 after haploidentical HCT.
Administration of the first infusion earlier than day +21 should be discussed with the Sponsor.

HSV-Tk infusion must not be infused in case of:

1. Infections requiring administration of ganciclovir or valganciclovir at the time of infusion.
2. GvHD requiring systemic immunosuppressive therapy
3. Ongoing systemic immunosuppressive therapy after haploidentical HCT
4. Administration of G-CSF after haploidentical HCT

HSV- Tk cells can be administered after and adequate wash out period (24 hours).

A delay in the treatment administration over 1 week must be justified in the patient's CRF.
The calculation of the dose of TK cells to be infused is based on the weight of the patient at the screening phase (i.e. before HCT), reported on documents of lymphocytoapheresis (such as Apheresis Shipping/Transduction Request Form); the patient's weight must also be provided in case of further request of transduction if more HSV-Tk doses are needed.
The dose of HSV-Tk cells of each infusion is $1 \pm 0.2 \times 10^7$ cells/kg.

5.6 Treatment duration

The planned treatment foresees the administration of up to 4 infusions of HSV-Tk donor lymphocytes with the purpose to induce immune reconstitution. Schedule and doses recommended for the phase

III, were obtained from the results of a Phase I-II trial (TK007) demonstrating safety and activity of the experimental product.

The HSV-Tk infusion will be discontinued only in presence of the following conditions:

1. CD3+ cells ≥100/µl (immune reconstitution)
2. Any grade 3-4 adverse event related to HSV-Tk infusion
3. Any grade 2 adverse event related that does not resolve to no more than grade 1 before the next infusion, for infusion that follow the first (unless investigator considers it to be in the best interest of patient to continue treatment).

The duration of the treatment will be related to the clinical outcome (achievement of immune-reconstitution).
For the long-term follow-up after HSV-Tk infusion(s) see section 10.5.4

5.7 Dose modification

A standard dose modification scheme is not applicable.

5.8 Treatment in case of disease relapse or progression

Leukaemia relapse or disease progression will be treated according to local Institutional policy, including chemotherapy, other antiproliferative agents, donor lymphocyte infusions or allogeneic transplantation from an alternative donor.

6 Study Assessments and Procedures

6.1 Pre HCT phase

6.1.1 Confirmation of eligibility (Randomization procedures)

Eligible patients who provided the informed consent to participate into the study will be randomly assigned to either treatment strategy before HCT, through a centralized randomization process using the following stratification factors: status of the disease at the time of transplantation (e.g., first or subsequent complete remission or relapse), ECOG performance status (0 or 1), and country.
In the experimental group A, patients will receive infusion of CD34+ cells plus a dose of T cells (approximately 1 x 10^4/Kg) followed by infusion of HSV-Tk genetically modified CD3+ cells.
In the control group B, the physician will choice if the patient will receive either infusion of CD34+ cells plus a dose of T cells (approximately 1 x 10^4/Kg) or unmanipulated haploidentical (bone marrow or peripheral blood) transplantation followed by high-dose cyclophosphamide.
Paper randomization lists, balanced within each stratum in blocks of varying size in random sequence, will be kept in MolMed headquarter. For each patient who undergoes screening, an Eligibility Screening Form (ESF) has to be filled in. The form is submitted to MolMed by fax/e-mail. The enrolment will be confirmed by fax/e-mail by the sponsor with the Confirmation of Enrolment Form, in which is reported the patient and donor (for arm A only) identification codes that must be used for the entire duration of the study, for the CRF and for all the other study forms. On the Confirmation of Enrolment Form will be also indicated the treatment arm (A: experimental arm; B: control arm). The ESF should be sent to the sponsor maximum 30 days before HCT.
The investigator or designee will use the Case Report Form (CRF). He/she will enter the assigned patient code number and treatment group allocation, reported in the confirmation of enrolment, in the appropriate place on each patient's CRF.

6.1.2 Screening phase

Written informed consent must be obtained prior to the patient and donor undergoing any study-specific procedures.
The screening phase will be performed maximum 30 days before haploidentical HCT and will include:

a. Patient and donor informed consents
b. Medical history and complete objective examination
c. Blood and chemistry: WBC (with formula), RBC, platelets, Hb, Hct, MCV, MPV, reactive C protein, albumin, albumin/globulin, IgG, IgA, IgM, glucose, AST, ALT, γGT, total bilirubin, LDH, alkaline phosphatase, creatinine, BUN, creatinine clearance (if appropriate) according to Cockroft-Gault formula (Appendix F), electrolytes (Na+, K+, Ca++)
d. Serology: CMV status, EBV-status, HCV, HBV and HIV
e. Evaluation of the neoplastic disease on bone marrow or peripheral blood
f. Cytogenetics and/or molecular tests of bone marrow and/or peripheral blood for markers of disease, according to center guideline
g. Left ventricular ejection fraction by MUGA scan or echocardiography
h. Spirometry with Diffusing Lung Capacity of Carbon Oxide (DLCO)
i. A serum or urine pregnancy test in women with reproductive potential
j. QTc interval by ECG
k. Donor's tests for: serology (CMV status, EBV status, HCV, HBV, HIV, Treponema Pallidum), mycoplasma (only Arm A), screening for Human transmissible spongiform

encephalopathy, including Creutzfeldt-Jakob disease, Human T-Lymphotropic virus, West Nile Virus*, Neisseria gonorrhoeae* and Chlamydia trachomatis*. The investigator shall select the donor in compliance with the 21 CFR 1271*, directive 2006/17EC** and applicable local guidelines.

*For US centers only. **For EU centers only.

The Donor's tests specific for patients randomized in arm A must be performed according to Appendix B.

6.1.3 Lymphocytoaphaeresis collection procedures for experimental Arm A

The donor lymphocytes will be collected before mobilization with G-CSF or marrow harvesting to avoid alterations in the immune repertoire, functionality, phenotype and cytokine production.

The collection will be via lymphocytoaphaeresis, upon donor informed consent.

The lymphocytoaphaeresis should be delivered to MolMed S.p.A. at least 20 days before the transplant.

Different timing of lymphocytoaphaeresis collection should be discussed with the Sponsor.

The lymphocytoaphaeresis (at least $10x10^9$ total WBC) is collected according to Institutional practice and sent to MolMed at 2-8°C, where it is processed in GMP area.

Clinical study centre should provide together with the lymphocytoapheresis bag, the declaration of biological tests performed within 30 days the collection (see appendix B) and the donor's blood group. Frozen commercial homogroup plasma will be used for transduction process.

Lymphocytoaphaeresis will be labelled indicating at least protocol code, donor code, site code or name, collection date, total volume, type of cells (i.e. lymphocytes) and according to the regulations.

Further details about procedures and forms are provided in the Study Manual.

In the event that a further lymphocytoaphaeresis is needed, it will be done at least 30 days after administration of G-CSF.

If indicated, in case of graft failure/rejection it will be possible to perform further HCT; in case of different donor a new lymphocytoaphaeresis will be sent to MolMed for transduction procedures. In this case, the investigator must inform the sponsor (using an appropriate form) in order to receive a new donor code.

6.2 Haploidentical transplantation (HCT) - Study day 0

6.2.1 Arm A: experimental group = haploidentical T-depleted HCT + HSV-Tk cells

Patients randomized in the experimental group, will receive a haploidentical T-depleted transplant followed by infusion(s) of HSV-Tk cells.

The first infusion will be administered between days +21 and +49 (see section 5.5 "Dosage schedule").

Administration of the first infusion earlier than day +21 should be discussed with the Sponsor.

The donor CD34 positive cells selection will be performed with the CliniMACS device.

Donor stem cells will be mobilised with G-CSF.

The graft composition should contain a recommended $7x10^6$ CD34+/kg stem cells and should be adjusted to contain approximately $1x10^4$ $CD3^+$/kg.

If indicated, in case of graft failure/rejection it will be possible to perform further HCT.

6.2.2 Arm B: control group = haploidentical HCT (T-cell depleted graft OR unmanipulated T-cell replete graft followed by high-dose cyclophosphamide and immunosuppressive therapy)

Patients randomized in the control group, will receive a haploidentical T-depleted transplant as reported above for arm A, OR an unmanipulated transplant (bone marrow or peripheral blood) followed by cyclophosphamide (50 mg/kg iv) on day +3 and +4.
In addition, after unmanipulated transplant, patients will receive filgrastim on day +4 (5 mg/kg/day by subcutaneous injection continuing until recovery of neutrophils to >1000/μL for 3 days) and, as GvHD prophylaxis, from day +5 tacrolimus or cyclosporine until day 180 and mycophenolate mofetil until day 35.
If indicated, in case of graft failure/rejection it will be possible to perform further HCT.

6.3 Post HCT phase - Arm A and Arm B

6.3.1 Disease evaluation

The disease evaluation will be collected after HCT (day O):

- monthly up to month 6 (±7 days)
- at month 9 and month 12 (±7 days)
- yearly until disease relapse or progression (±1 month)

The following disease examinations will be performed:

- Morphology (bone marrow or peripheral blood)
- Confirmation of mixed or full chimerism (evaluation of the degree of chimerism between donor/host, according to institutional clinical practice, on bone marrow or peripheral blood)
- Cytogenetic and/or molecular and/or other tests, according to institutional clinical practice (bone marrow or peripheral blood).

After disease relapse or progression, patients will be followed up for further antileukemia treatments, survival (see section 6.3.5), safety (see section 6.3.7) and for patients treated with HSV-Tk cells, also for RCR (see section 6.4.2) and Long Term Follow-up (see section 10.5.4).

6.3.2 Laboratory assessment

The laboratory assessments will be performed after HCT (day O), at the following time-points:

- at day 15 of 1st month (±2 days)
- monthly up to month 6 (±7 days)
- at month 9 and month 12 (±7 days)

Blood and chemistry assessment includes: AST, ALT, γGT, total bilirubin, LDH, WBC (with formula), RBC, platelets, Hb, Hct, MCV, MPV, creatinine, CMV PCR or antigenemia. Exception will be made for the 1st examination after HCT that will be performed as indicated in the screening phase.

6.3.3 Physical examination

Physical examination will be performed after HCT (day O), at the following time-points:

- monthly up to month 6
- at month 9 and month 12

The following assessments will be performed:

- Vital signs (blood pressure, pulse rate)
- Body weight
- ECOG performance status (see appendix E)
- Evaluation of clinical signs of GvHD

6.3.4 Functional Studies

Functional studies, aimed at quantifying and characterizing immune reconstitution are planned for patients enrolled in arm A and arm B.
Studies involving the identification of genetically modified lymphocytes (LNGFR marking) will be performed only in patients randomized for Arm A.

6.3.4.1 Immune phenotype analysis

The number of circulating $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ cells will be quantified and used to evaluate immune-reconstitution. These analyses shall be performed with flow cytometrically using an immunological gating strategy; a technique similar to the ISHAGE (International Society of Hematotherapy and Graft Engineering) sequential gating protocol used for the enumeration of CD34+ stem cells[23]. Immunological gating of CD45+ leukocytes minimizes interference from debris which antibodies can bind non-specifically. The single platform method will be used to determine absolute counts. This method employs an internal standard of fluorescent microbeads which enables the direct enumeration of cells bearing each phenotype.

Immune phenotype analysis to quantify circulating lymphocytes will be performed after hematologic engraftment of haploidentical HCT in both arms, as follows:

- weekly up to IR achievement

and after IR:

- monthly up to 6 months (±7 days)
- at month 9 and month 12 (±7 days)

Aliquots of whole blood specimens for both arms will be stained with the following panel of monoclonal antibodies
CD3
CD3/CD4
CD3/CD8
CD45/CD3/CD19
CD45/CD16/CD56
CD45/LNGFR/CD3 (Arm A only)

CD45/CD3/CD4/CD8 (at all time points with CD3 ≥ 100/µl)
CD45/LNGFR/CD4/CD8 (Arm A only; at all time points with LNGFR > 20/µl)

In addition, in the arm A, in presence of GvHD related to HSV-Tk cells and treated with GCV/VCV, immune phenotype analysis will be performed at the following time-points:

- before GCV/VCV treatment

- 4 days after the beginning of GCV/VCV treatment
- 1 day after the discontinuation of GCV/VCV treatment

Further optional analyses regarding the functional characterization of immune reconstitution have been listed in Appendix F.
Analysis and reporting of these data will be handled separately from the clinical study report.

6.3.5 Survival follow-up

After the first year from HCT or after disease relapse/progression, patients of both arms will be followed on an every 6-month basis for further anti-leukemia treatments and survival.
For patients of both arms who withdraw their consent, every effort must be made to document if a subject withdrew consent from study treatment or for all study procedures and follow-up. Unless specifically stated by the patient who withdrew consent for follow-up, further anti-leukemia treatments and survival follow-up will continue as indicated.

6.3.6 Quality of life assessment

An assessment of the quality of life (QoL) by questionnaire (see Appendix J) will be performed:

- before HCT
- at month 9 and 12
- yearly after HCT until disease relapse

The objective of quality of life questionnaire will be to summarize and evaluate treatment group differences in patient convenience and satisfaction.

6.3.7 Safety

The safety study data will be collected as follows:

- Starting from HCT, only unexpected serious adverse events (SAEs) and study endpoints (graft failure/rejection, disease relapse, grade II-IV acute and all chronic GvHD, and death) will be collected as SAE until day 21.
- From day 21 through day 180, all adverse events (AEs) will be collected
- In case of HSV-Tk infusion before day 21, AEs have to be collected from the day of first infusion through day 180 (or at least 30 days after last infusion, whichever is longer).
- Any event that was present before day 21 and that remains unchanged or improved should not be recorded as an AE. If the event gets worse on day 21, this should be considered an AE.
- Related AE/SAE, infections and GvHD not resolved at day 180 will be followed through the resolution. All other AEs will be closed as not resolved or unknown and the end date will be not available
- From day 181 (or 30 days after last infusion of HSV-Tk), only related SAEs and study endpoints will be collected (as SAE) through end of study.

In case of disease relapse AE/SAE collection will be stopped with the following exception:

- Related AE/SAE and infections not resolved when the relapse occurred will be followed through resolution
- Death (as study end point) and related SAE will be collected as SAE through end of study
- Only for patients in arm A, AE/SAE unrelated will be collected until day of relapse or 30 days after the last HSV-Tk infusion (whichever happens later)

The table below summarizes the data safety collection:

	Day 0 (HCT)→ Day 21[1]	Day 21[1]→ Day 180[2]	Day 180 → EoS[3]	AFTER RELAPSE	
				Arm A	Arm B
Study endpoints*	X	X	X	Only Death	
Unexpected SAE	X				
AE/SAE Unrelated		X		X[4]	
AE Related		X	X[5]	X[6]	
SAE Related		X	X	X	
Infection		X	X[5]	X[6]	

[1] for ARM A=day of first infusion (if anticipated)
[2] for ARM A= day 180 or 30 days after last infusion (whichever happens later)
[3] EoS= End of Study
[4] until date of relapse or 30 days after last infusion (whichever happens later)
[5] only if not resolved at day 180
[6] only if not resolved when the relapse occurred
*Study endpoints (graft failure/rejection, disease relapse, GvHD, death) have to be reported as Serious Adverse Events on SAE Form

For further details on safety reporting see also section 10.3.

6.3.7.1 Treatment of GvHD related to HSV-Tk cells

The classification of acute and chronic GvHD are reported in Appendix C and D, respectively.
If at any time during the study, a grade of acute GvHD equal or greater than 2 or a chronic GvHD occurs, related to HSV-Tk cells, the patient will be treated with ganciclovir at a dose of 10 mg/kg/day divided into 2 administrations, or valganciclovir 900 mg twice per day orally for 14 days.
For more detailed information about the ganciclovir (GCV) or valganciclovir (VCV) administration, the investigator should refer to the related "Summary of Product Characteristics".
In case of GvHD progression after 3 days of treatment with only GCV/VCV, a standard immunosuppressive therapy (including corticosteroids, cyclosporine, mycophenolate mofetil, tacrolimus, etc) will be added as per standard practice and according to clinical center protocol.
HSV-Tk cells could be administered after a 24-hour discontinuation period of GCV/VCV or immunosuppressive therapy.

Immune phenotype analysis (see Section 6.3.4.1) will be performed for patients treated with GCV/VCV, in order to monitor the control of GCV/VCV on GvHD, at the following time points:

- before GCV/VCV treatment
- four days after the GCV/VCV beginning
- one day after the discontinuation of GCV/VCV treatment.

6.3.7.2 Treatment of GvHD not related to HSV-Tk cells

If acute GvHD or chronic GvHD develop, a standard immunosuppressive therapy will be used according to center guideline.

6.4 Specific assessments for Arm A (centralized analyses)

6.4.1 Real time PCR-TK

PCR analysis for HSV-Tk aims at verifying the presence of transduced donor lymphocytes, will be performed at the following time points:

- baseline (i.e. immediately before 1st HSV-Tk infusion)
- monthly starting from the 1st HSV-Tk infusion up to 6 months after last administration (± 7 days)

6.4.2 Analysis of retrovirus competent for replication (RCR)

RCR search will be carried out using molecular tests (Q-PCR env) according to the following time schedule:

- at baseline (i.e. immediately before 1st HSV-Tk infusion)
- at 3 months after 1st HSV-Tk infusion (± 7 days)
- at 6 months after 1st HSV-Tk infusion (± 7 days)
- at 1 year after 1st HSV-Tk infusion then (± 1 month)
- yearly for at least 5 years (± 1 month)

In case the samples collected during the first year were always negative, the subsequent yearly samples will be taken but not analysed; in case of one or more positive samples, the culture test will be performed for confirmation.

6.4.3 Sampling procedures

For each test, at least $5x10^6$ PBMC post Ficoll are requested, that are obtained in general from a sample of 10 ml blood. Double volumes are generally necessary at baseline to obtain 5×10^6 PBMC. The frozen samples will be sent in dry ice to Sponsor.
All documentation related to collection, shipment, delivery, receipt, storage and destruction must be filed and the related information recorded.
Details about procedures and forms are specified in the Study Manual.

6.5 Study Flow Chart

	Pre-Randomization Screening Phase	Study Treatment Phase (for both experimental and control arm)			Follow up Phase	
		Haplo-HCT	1 to 6			
Study Month	**- 1**	**0**			**9 and 12**	**24 to end of study**
Study Day	**-30 to -1**	**0**	**1 to 180**		**181 to 365**	**366 to end of study**
			Weekly	**Monthly**	**Every 3 months**	**Yearly**
Informed Consent[1]	X[2]					
Demographic Data	X					
Medical History	X					
Leukemia Treatment History	X					
Pregnancy Test (if applicable)	X[2]					
Serology	X					
Functional tests (DLCO, LVEF and QTC)	X[2]					
Concomitant Treatments	X		X	X		
Physical Examination[3]	X[2]			X	X	
Hematological & biochemical exams	X[2]		X[4]	X	X	
Study Treatment Administration				X[5]		
Immunophenotype (quantification)			X[6]	X[7]	X[7]	
Disease evaluation	X[2]			X	X	X[8]
QoL Questionnaire	X				X	X[8]
Real Time PCR-TK (arm A only)				X[9]		
RCR analysis (by PCR) (arm A only)				X[10]		X[10]
Safety		On an ongoing basis				
Survival follow-up						X[11]
Long-term follow up (arm A treated only)						X
Further antileukemia treatment						X[11]

[1] Informed consent must be obtained before any study specific screening procedures are performed.
[2] Data used by investigator to evaluate patients' eligibility.
[3] Including evaluation for clinical signs of GvHD
[4] To be performed at day 15 of 1st month from HCT
[5] 1st infusion from day +21 to +49, the subsequent every month up to a maximum of 4 infusions
[6] To be performed from engraftment to immune reconstitution
[7] To be performed after IR, monthly for 6 months and then at M 9 and M12
[8] To be performed until disease relapse or progression
[9] To be performed immediately before the 1st infusion and monthly starting from the first HSV-Tk infusion up to 6 months from last administration
[10] To be performed immediately before the 1st infusion and at 3-6-12 months after first TK infusion and then yearly for 5 years
[11] To be assessed every 6 months after the first year from HCT or after disease relapse/progression

7 Pharmaeconomics

An exploratory pharmacoeconomic analyses will be performed on medical care utilization (MCU). The objective will be to summarize and evaluate treatment group differences in total resource use, and more specifically, in resource use associated with diagnosis, monitoring and treatment of relevant events (i.e., acute and chronic GvHD, infectious episodes, etc.).
Pharmacoeconomic data may be combined with other data such as cost data or other clinical parameters in the production of a final pharmacoeconomic report.
The analysis and reporting of resource use data will be handled separately from the clinical study report.

8 Response evaluation criteria in Acute Leukaemia

Response to treatment will be assessed according to standard international criteria[24,33].
Evaluation of disease status will be performed accordingly to the schedule reported in Chapter 6.
For the assessment of outcome, relapse is defined as bone marrow blasts ≥ 5%; or reappearance of blasts in the blood; or development of extramedullary disease. In cases with low percentages (5-10%), a repeat marrow should be performed to confirm relapse. Relapse will be evaluated and classified for each patient by Investigator.

9 Statistical considerations

9.1 Statistical design

This is a randomized (with a 3:1 allocation experimental group: control group), 2-arm, open-label, multicenter, multinational, phase III study.
The unbalanced randomization has been chosen in order to enhance the safety database and also to make the study more attractive to patients and/or investigators (*ICH E10 "Choice of Control Group in Clinical Trials, CPMP/ICH/364/96"*).
Eligible patients who provided the informed consent to participate into the study will be randomly assigned to the either treatment strategy before HCT, through a centralized randomization process using the following stratification factors: status of the disease at the time of transplantation (first or subsequent complete remission or relapse), ECOG performance status (0 or 1) and country. Random lists, balanced within each stratum in blocks of varying size in random sequence, will be prepared and kept at the Study Coordination Center in MolMed headquarter.
The study will be conducted according to GCP, and will undergo formal monitoring.
All randomised patients will be followed until death or study closure, whichever first.
The primary endpoint of the study is disease-free survival (DFS), which allows to assess all the potential effects of the experimental treatment on patients' outcome, including infectious mortality, GVHD-related mortality, as well as incidence of relapse.

9.2 Study endpoints

9.2.1 Primary endpoint: Disease-free survival (DFS)

The primary endpoint of the study is disease-free survival (event-free survival, EFS) that will be measured for all patients from the date of randomization (regardless of disease status at HCT) until the date of relapse (or progression), or death from any cause, whichever occurs first.
Patients without an event will be censored at the last time known to be alive and free of relapse. Patients without any follow-up information will be censored at the date of randomization.

9.2.2 Secondary endpoints

a. Overall Survival (OS) will be measured for all patients from the date of randomization until death from any cause. Patients without any follow-up information will be censored at the time of randomization. Patients alive and lost to follow-up before study closure will be followed by phone contacts and by contacting census offices for assessing their vital status at study closure. In case of failure, they will be censored at the last follow-up examination, but reported and analysed for possible biases.
b. Non-relapse mortality (NRM) will be defined for all patients as any death without previous occurrence of a documented relapse (or progression), which is a competing event. Patients alive without relapse (or progression) will be censored at last contact.
c. Chronic GvHD-free/relapse-free survival (GRFS) defined as the time from the date of randomization to chronic GvHD, relapse/progression or death from any cause, whichever occurs first.
d. Time to immune reconstitution (IR) will be defined as the time to reach a level of circulating $CD3^+ \geq 100/\mu l$ for two consecutive observations, starting from the date (study day 0) of transplantation.

e. Engraftment will be defined as the persistent blood cells count above a predefined level (ANC ≥ $1x10^9$/L per 3 consecutive days with evidence of donor haematopoiesis; platelets ≥ $50x10^9$/L, unsupported by transfusions, for 7 days), and is computed from the date (day 0) of transplantation
f. Cumulative incidence of grade 2, 3, or 4 acute GVHD (aGvHD), diagnosed and graded according to standard criteria, will be computed from the date (study day 0) of transplantation.
g. Cumulative incidence of chronic GvHD (cGvHD) diagnosed and graded according to standard NIH consensus criteria, will be computed from the date (study day 0) of transplantation.
h. Duration of GvHD episodes computed from the date of start to the date of resolution and duration of immunosuppressive treatments administered for controlling GvHD.
i. Cumulative incidence of relapse (CIR) will be defined on the basis of morphologic evidence of leukaemia in bone marrow or other sites. The events are relapses (or progressions). Patients alive without relapse (or progression) will be censored at last contact. Death without experiencing a relapse (or progression) will be considered as competing event.

Safety: all safety parameters will be analysed and presented in terms of listings and summary tables. Adverse events and laboratory parameters will be assessed according to the CTC-AE v4.02.
Adverse events will be displayed in standard frequency tables.
For laboratory parameters, descriptive summary tables of change from day 21 over time based on SI units will be produced. Summary tables of the worst grades according to CTC-AE v4.02 observed during the treatment will be presented.
Descriptive summary tables of change from day 21 over time will be provided for vital signs parameters.

A summary of the criteria to define the outcomes in survival analysis is listed in the following table:

Table 2. **Summary of the criteria to define the outcomes in survival analysis**

Outcome	Event	Censored cases	Competing events	Population
Disease-free survival	Relapse (or progression) or death regardless of cause	Patients alive without relapse (or progression) at last contact	-	All patients
Overall survival	Death regardless of cause	Patients alive at last contact	-	All patients
Cumulative incidence of relapse	Relapse (or progression)	Patients alive without relapse (or progression) at last contact	Death without evidence of relapse (or progression)	All patients
Non-relapse mortality	Death without previous relapse (or progression)	Patients alive without relapse (or progression) at last contact	Relapse (or progression)	All patients
GRFS	Chronic GvHD, relaps (or progression) or death regardless of cause	Patients alive without chronic GvHD or relapse (or progression) at last contact	-	All patients

Engraftment	Persistent blood cells counts above the predefined level	Patient alive with no recovery at last follow-up	Death and relapse (or progression) before recovery	All patients
Time to immune reconstitution	Persistent CD3+ cells above the predefined level	Patient alive with no recovery at last follow-up	Death and relapse (or progression) before recovery	All patients
Acute GvHD	aGvHD	Patient alive with no occurrence of aGvHD	Death and relapse (or progression) without aGvHD	All patients
Chronic GvHD	cGvHD	Patient alive with no occurrence of cGvHD	Death and relapse (or progression) without cGvHD	All patients

9.3 Statistical analyses

The primary study analysis will be based on the Intention To Treat (ITT) approach, in that all randomised patients, regardless of their eligibility and compliance to the assigned treatment and to the follow-up protocol will be included in the analyses and considered in the group assigned at randomization. For explorative purpose, efficacy analyses will be repeated on the Standard Population (STDP) that includes all randomised patients who have received at least one haploidentical HCT.

9.3.1 Primary study analyses

The log-rank test will be used to compare disease-free survival (DFS) in the two treatment arms with stratification for ECOG PS and disease status at randomization. The choice of not including the other randomization stratification factor, i.e. country, among the analysis stratification factor is dictated by the risk of a loss of power, since the primary endpoint is a time-to-event variable, and the number of strata for a fully stratified analysis would be rather large when compared to the number of the enrolled patients. Kaplan-Meier curves will be displayed and 1-year DFS with confidence limits will be given. Cox regression analyses will be performed. Covariates included in these analyses will be, among others, age, gender, disease, ECOG PS, disease status at randomization and country.

9.3.2 Secondary analyses

Overall survival (OS) curves will be estimated by the Kaplan-Meyer method and compared using the log-rank test.
In order to take into account the plausible lack of independence between non-relapse mortality (NRM) and cumulative incidence of relapse (CIR), a standard competing risks analysis will be performed, where the cumulative incidence of events, computed as 1-cumulative Kaplan Meier curve, will be partitioned into its components, the crude cumulative incidence of non-relapse mortality and the crude cumulative incidence of relapse. The Gray test[34] will be used to compare the sub-distribution functions of the two competing events in the two treatment groups.
Time to immune reconstitution, incidence of acute GvHD, chronic GvHD and GvHD resolved with immunosuppressive agents and incidence of engraftment will be compared in two groups by means of five separate competing risk analyses. In each of these analyses, the competing events will be death and relapse before the event of interest. The Gray test will be used to compare the distribution of events of interest in two treatment arms.

9.4 Sample size

The primary study endpoint is disease-free survival (DFS). The 1-year DFS following standard haploidentical HCT (with either infusion of CD34+ cells plus a dose of T cells (approximately $1x10^4$/Kg) or unmanipulated bone marrow or peripheral blood transplantation followed by high-dose cyclophosphamide) reported in the available studies averages at approximately 30%, and this figure can be used as a reasonable estimate of the cumulative incidence of DFS expected in the control group of the present study. The estimated 1-year DFS in the phase II study (TK007) evaluating the add back of HSV-Tk donor lymphocytes strategy was 52%, corresponding to a hazard ratio (HR) of 0.55, when compared to historical controls. In order to detect with α=0.05 (2-sided) and β=0.20 a hazard ratio of 0.55, 96 events (relapses or deaths) need to be observed in the 2 groups combined. To this aim, 170 patients (127 in experimental arm and 43 in control arm) need to be enrolled and followed for at least one year.

10 Safety

10.1 Definitions

An **Adverse Event** (**AE**) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease absent before day 21 and temporally associated with the use of the medicinal product, whether or not considered related to the medicinal product.

Any event that was present before day 21 and that remains unchanged or improved should not be recorded as an AE. If the event gets worse on day 21, this should be considered an AE.

An **Adverse Drug Reaction** (**ADR**) is any untoward and unintended response to an investigational medicinal product related to any dose administered.

An **Unexpected Adverse Drug Reaction** is any adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure).

A **Serious Adverse Event** (**SAE**) or a **Serious Adverse Drug Reaction** (**SADR**) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definitions above. These situations should also usually be considered Serious Adverse Events.
Any clinically significant laboratory test value that meets the definition of a SAE must be reported as a SAE.

10.2 Criteria of evaluation

All adverse events will be recorded according to CTC version 4.02 (CTC reference: http://ctep.cancer.gov/reporting/ctc.html) on the case report forms (CRFs); the investigator will decide if those events are drug related and his/her decision will be recorded on the forms for all adverse events. The grading of acute and chronic GvHD are reported in Appendix C and D, respectively.
The adverse events not listed on the NCI CTC grading system will be graded on a five points scale and reported in detail on the CRF, as indicated:

Mild = Grade 1	Discomfort noticed but no disruption of normal daily activity
Moderate = Grade 2	Discomfort sufficient to reduce or affect daily activity
Severe = Grade 3	Inability to work or perform normal daily activity
Life-threatening = Grade 4	Represents an immediate threat of life
Death = Grade 5	Death related to the adverse event

Relationship of the adverse event to the treatment should also be assessed using the foreseen categories for determining relationship:

Not related	The adverse event is clearly not related to the investigational product or HCT
Related to HSV-Tk	The adverse event is clearly related to the investigational product
Related to HCT	The adverse event is clearly related to HCT
Unknown	This category should be used when it is difficult to strongly appoint if the adverse event is drug related or not, or information about it is unavailable, missing or incomplete

10.3 Reporting procedures

10.3.1 Adverse events

All AEs will be collected from day 21 through day 180 or at least 30 days after last dose of HSV-Tk, whichever will be longer.
In case of HSV-Tk infusion before day 21, AEs have to be collected from the day of first infusion through day 180 (or at least 30 days after last infusion, whichever is longer).
Hematological and biochemical adverse event have to be reported, unless investigator consider them not clinically significant.
After disease relapse, AE collection will be stopped (except for AE related or patients in arm A, for whom the AE collection have to be continued until 30 days after last HSV-Tk infusion).

10.3.2 Serious Adverse Events

Only unexpected Serious Adverse Events (SAEs) and study endpoints (graft failure/rejection, disease relapse, grade II-IV acute and all chronic GvHD, death) will be collected from HCT to day 21 (as SAEs).
From day 21 through day 180, all SAEs and study endpoints (to be reported as SAEs) will be collected.
Only related SAEs and study endpoints will be collected (as SAE) from day 181 (or 30 days after last infusion of HSV-Tk) through end of study (i.e. end of long term follow-up).
All grades 3-5 infusion reactions, late graft rejection and grades 3-4 acute GvHD will be reported in an expedited fashion in addition to the unexpected SAEs.
After disease relapse, SAE collection will be stopped (except for patients in arm A, for whom the SAE collection have to be continued until 30 days after last HSV-Tk infusion) and only related SAEs and deaths will be collected (as SAEs) through end of study.

All the SAE must be reported to MolMed S.p.A. **within 24 hours** of the initial observation of the event.
This must be done by faxing or emailing a specific SAE form to the following address:

Antonio Lambiase, MD
Qualified Person for PharmacoVigilance
(QPPV)
Phone +39 02 21277 232
Mobile +39 334 6694051
Fax +39 02 21277 239
E-mail safety@molmed.com

It is the responsibility of the investigators to promptly send a safety report to the Ethics Committee according to the local regulation.

Hospitalization for elective treatment of a pre-existing condition, including also the administration of therapy for CMV infections, should NOT be reported as AE/SAE unless worsening or complications.

It should be recognized that Serious Adverse Drug Reactions (SADR) which have not been previously documented in the Investigator's Brochure, or which occur in a more severe form than anticipated (i.e. they are "unexpected" by nature or severity), are subject to rapid reporting to the Regulatory Authorities by the Sponsor.

10.4 Procedures to be followed in the event of pregnancy

If a female patient or the partner of a male patient becomes pregnant during the active treatment phase or within 6 months from discontinuation of the study drug, she/he must immediately inform the investigator.
In case of pregnancy the female patient will be withdrawn from the active treatment.
The investigator should report the event of pregnancy within 24 hours to the Sponsor, using the form for Serious Adverse Events and the additional specific form (Clinical Trial Pregnancy Follow up Reporting Form).
The investigator should counsel the patient and discuss the risk of continuing with pregnancy and the possible effects on the fetus. The patient will be monitored until the conclusion of the pregnancy.

10.5 Risk management plan

As part of the Conditional Marketing Authorisation (CMA) granted in August 2016, by the European Medicines Agency (EMA) for the medicinal product (www.ema.europa.eu/EPAR/Zalmoxis), the following events have been classified as important identified risks: GvHD, severe systemic infection, CMV and EBV reactivation, febrile neutropenia, hepatic failure, concomitant administration of ganciclovir/valganciclovir; while the following events have been classified as important potential risks: concomitant immunosuppressive therapy, development of RCR, carcinogenicity, genotoxicity, late complications as malignancies or autoimmunity, immunological events (antibody formation), DMSO-related side effects, local and systemic donor reaction and failure of GvHD treatment with ganciclovir.

10.5.1 RCR

It is well known that lymphocytes can be efficiently transduced by retroviral vectors. A number of different retroviral vectors based on the Moloney Murine Leukaemia Virus (MolMLV) backbone have been engineered and used in gene transfer into human hematopoietic cells. Although vectors are

designed to be replication defective, recombination events during vector production could lead to the generation of replication competent retroviruses (RCR). While replication defective vector carry risk, the risk of insertional mutagenesis is believed to be greater if RCR is present, since ongoing viral infection is likely to result in a much greater number of insertional events. Data obtained in primates have demonstrated RCR contaminating retroviral vector preparations could cause malignancy, but tumours may develop only in primates that are very immunodepressed[35,36] .The technologies available today for the RCR monitoring in both the packaging cell lines and the ex vivo engineered tissues allow for a very effective quality control for RCR presence, thus virtually eliminating this risk even in immunodepressed patients. The clinical trials performed-up to now using engineered lymphocytes via retroviral vectors have never recorded any toxic reactions or side effects due to the retroviral vector, the engineering procedure or the exogenous protein.
RCR arises by recombination between the vector and viral genes, and was frequently detected in early versions of vector packaging cell lines in which all the viral genes (gag/pol/env) were expressed from a single plasmid. By segregating gag-pol and env genes onto separate plasmids and minimizing homology between vector and packaging sequences, the rate of RCR development has been substantially decreased, but not eliminated.
In addition, the marked decrease in RCR generation resulting from decreased homologous recombination has resulted in rare recombination between vector and cellular sequences leading to RCR, or rescue of endogenous viruses especially when murine-based packaging cell lines are utilized. To date, there have been no documented exposures of patients treated with gene therapy to RCR. The clinical trials performed up to now using engineered lymphocytes via retroviral vectors have never recorded any toxic reactions or side effects due to the retroviral vector, the engineering procedure or the exogenous protein (except for lymphocytes expressing TCR and CAR)[37,38]. HSV-Tk cells infusion have shown to not to affect biological and function of transplanted T cells[39]. Analysis of 102 independent transductions of human peripheral lymphocytes with two different vectors (SFCMM-3 and SFCM) encoding the same HSV-Tk and ΔLNGFR detected no change in the expression of markers of lineage, activation or adhesion, or in the proliferative capacity of T cells, as assayed by limiting dilution after polyclonal in vitro stimulation. All cells remained strictly dependent on IL-2 for growth and survival, and the addition of potentially stimulatory doses of 50- 100 ng/ml of nerve growth factor did not induce cell proliferation, expression of the CD25 activation marker or secretion of tumor necrosis factor, thus providing direct evidence that ΔLNGFR is a safe and inert gene marker for T lymphocytes even in the presence of high concentrations of the ligand. Most importantly, no toxicity or other adverse effects have been associated with the clinical use of HSV-Tk and ΔLNGFR. Treatment of 31 patients with donor lymphocytes transduced with two different vectors (SFCMM-2 or SFCMM-3) encoding the same HSV-Tk and ΔLNGFR resulted in engraftment (up to 40% of circulating mononuclear cells) and long-term persistence (>80 months) of transduced cells. No acute or chronic adverse or toxic events related to the gene transfer procedure or to transgene expression were observed during these trials, which involved infusion of >1011 cells generated by >50 independent transductions.[40] The HSV-Tk and ΔLNGFR therapeutic approach utilizes donor lymphocytes that are also equipped with an efficient suicide system, and no evidence of insertional oncogenesis has been recorded over long-term clinical experience with HSV-Tk-treated lymphocytes. In case of one or more positive samples collected for the study, the case(s) will be considered a SUSAR and therefore subject to rapid reporting to the Regulatory Authorities by the Sponsor.

10.5.2 Safety monitoring

The safety will be monitored during the study according to the following schedule:

Blood and chemistry examination (for all patient) at day 15 of 1st month, monthly for 6 months (±2 days) and then at month 9 (±2 days) and 12 (±2 days) after HCT. The examination foresees: AST, ALT, γGT, total bilirubine, LDH, WBC (with formula), RBC, platelets, Hb, Hct, MCV, MPV, creatinine, CMV PCR or antigenemia). Exception will be made for the 1st examination after HCT that will be performed as indicated in the screening phase (for details see § 6.1.2).

RCR test (for patients in Arm A only) at baseline (i.e. immediately before 1st HSV-Tk infusion), 3 months, 6 months and 1 year after 1st HSV-Tk infusion, and then yearly for at least 5 years (for details see § 6.4.2).

Long term follow-up (for details see § 10.5.4).

10.5.3 RCR monitoring during manufacturing

RCR may develop at any step during manufacturing from development of the initial master cell bank through production of the retroviral vector supernatant. In addition, the growth of ex vivo transduced cells provides the potential for amplification of any RCR contaminant which may be below the level of detection in the retroviral vector supernatant.

The RCR test should be performed at the following time point:

- Vector Producer Cell Master Cell Banks (one time testing): both Vector Producer Cells and the culture are tested by a cultural RCR assay.
- Testing of Retroviral Vector Supernatant Product and End of Production Cells are tested by a cultural RCR assay
- Ex-vivo Transduced lymphocytes, which according to the manufacturing protocol are cultured for more than 4 days, are tested by a molecular RCR assay. (*Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors FDA Guidelines-11.2006).*

10.5.4 Long term follow-up after HSV-Tk treatment

This study foresees a long term follow-up period of 15 years after the first year from HCT, as follows:

- Every year a general health evaluation, including new/recurrent malignancies, neurologic disorders, hematologic disorders, autoimmune disorders, unexpected medical problems and hospitalizations, will be collected by a specific questionnaire (see appendix K)
- For the first 5 year a blood sample for RCR test will be collected from patients treated with HSV-Tk cells, as specified in section 6.4.2.
- The long term follow up of patients treated with HSV-Tk cells has the aim to detect early or delayed signals of adverse reactions, to prevent clinical consequences of such reactions and to ensure timely treatment and to gain information on the long-term safety of the treatment.

For these reasons, also patients, who have interrupted the treatment, the long term follow up should be maintained, as well as, every efforts should be done for patients withdrew from the study, subject to the consent of the subject. Patient alert cards that inform treating physicians about the product used will be provided to patients. These alert cards, which should have been previously approved by the Ethics Committee, should contain as minimum the name of the patient, the investigator contact number and information regarding the medical treatment received.

11 Ethics and General study administration

11.1 Ethical aspects

The responsible investigator will ensure that this study is conducted in full conformance with either the principles of the "Declaration of Helsinki" (as amended in Tokyo, Venice, Hong Kong, South Africa, Edinburgh, Seoul and Fortaleza) or the laws and regulations of the country in which the study was conducted, whichever affords the greater protection to the individual.
The protocol has been written and the study will be conducted in conformity to the "Guideline for Good Clinical Practice" (recommended for adoption at step 4 of the ICH process on 1 May 1996 and on 10 June 1996 by the ICH Steering Committee and acknowledged as ministerial decree, on 15 July 1997, by the Italian Ministry of Health) and “Detailed guidelines on good clinical practice specific to advanced therapy medicinal products”.

11.2 Independent Ethics Committees/Institutional Review Board

This protocol and any accompanying material provided to the subject, such as subject information sheets or descriptions of the study used to obtain informed consent, will be submitted by the investigator to an Ethics Committee.
Approval from the Committee must be obtained before starting the study and should be documented in a letter to the investigator specifying the date on which the Committee met and granted the approval.
Any modifications made to the protocol after receipt of the Independent Ethics Committee approval must also be submitted by the Sponsor to the Committee in accordance with local procedures and regulatory requirements.
When no local review board exists, the Sponsor is expected to submit the protocol to a regional committee.

11.3 Informed consent

All patients and donors will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she will be exposed, and the mechanism of treatment allocation. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.
It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient’s subsequent care.
It is responsibility of the investigator or a person designated by the investigator (if acceptable by local regulations) to obtain written informed consent from each subject participating in this study before his/her registration in the trial.
For patients and donor unable to read and write, impartial witnesses should be present during the entire informed consent discussion. After the subject and representative have orally consented to participation in the trial, the witness’ signature on the form will attest that the information in the consent form was accurately explained and understood.
In the case of a minor donor (<18 years), parents or legal representatives must give permission by signing a specific informed consent form and according to local laws.
If new safety information results in significant changes in the risk/benefit assessment, the consent form is reviewed and approved, if necessary. All patients, including those already being treated,

should be informed of the new information and give their consent to continue the study after receiving a copy of the revised informed consent form.

11.4 Conditions for modifying the protocol

All protocol modifications must be submitted to the appropriate regulatory agencies: Ethics Committee (EC/IRB) and Competent Agency (CA) in accordance with local requirements. Approval must be awaited before any changes can be implemented except for changes necessary to eliminate an immediate hazard to trial subjects or when the change(s) involve only logistical or administrative aspect of the trial (change in monitor, change in telephone-fax number).

11.5 Conditions for terminating the study

Both the Sponsor and the investigator reserve the right to terminate the study at any time.
If this should be necessary, both parties will arrange the procedures on an individual study basis after review and consultation. In terminating the study MolMed and the investigators will assure that adequate consideration is given to the protection of the patient's interest.

11.6 Study documentation: CRF and record keeping

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into 2 different categories:

1. Investigator's study file
2. Subject clinical source documents

The investigators' study file will contain the protocol/amendments, CRF (Case Report Form) and query forms, regulatory approvals (EC/IRB and CA) with correspondence, sample of informed consent, drug/product records, staff CVs and authorization forms and other appropriate documents/correspondence.
Subject clinical source documents would include patient hospital/clinic records, physician's and nurse notes, appointment book, original laboratory reports, ECG, EEG, x-ray, pathology and special assessment reports, signed informed consent forms, consultant letters and subject screening and enrolments logs.
All traceability records should be kept for a minimum of 30 years after the end of study by each party: lymphocytoapheresis establishments/procurement, manufacturer, Sponsor and investigator/clinical site, as outlined in the annex of "Detailed guidelines on good clinical practice specific to advanced therapy medicinal products" and in Regulation 1394/2007
After that period of time the documents may be destroyed, subject to local regulations and sponsor agreement.
Should the investigator cannot guarantee this archiving requirement at the investigational site for any or all the documents special arrangements must be made between the investigator and MolMed to store these in a sealed container outside of the site so that they can be returned sealed to the investigator in case of regulatory audit where source documents are required for the continued care of the patient appropriate copies should be made for storing outside of the site.
Unless other local law requires archiving for a longer period, the Sponsor and the investigator shall archive the content of the clinical trial master file for at least 25 years after the end of the clinical trial. However, the medical files of subjects shall be archived in accordance with national laws

11.7 Source documents and background data

The investigator shall supply the Sponsor on request with any required background data from the study documentation or clinic records. In case of special problems and/or governmental queries or request for audit inspections it is also necessary to have access to the complete study records provided that patient/donor confidentiality is protected.

11.8 Audits and inspections

The investigator should understand that source documents for this trial should be made available to appropriately qualified personnel from MolMed or its designees or to health authorities.

11.9 Case Report Forms

For each patient enrolled an electronic case report form (eCRF) must be completed and signed by the principal investigator or one of his/her authorized staff members.
This also applies to records for those patients who fail to complete the study. If a patient withdraws from the study the reason must be noted on the CRF.
If a patient is withdrawn from the study because of a treatment limiting adverse event thorough efforts should be made to clearly document the outcome.
The investigator should ensure the accuracy, the consistency with source data. It's recommended to fill in the eCRF on an ongoing basis and submit the data no later than 7 working days after a patient's last visit. Manuals on eCRF use and data collection are available on website of eCRF.

11.10 Monitoring the study

It is understood that the responsible MolMed monitor or designee will contact and visit the investigator regularly and will be allowed on request to inspect the various records of the trial provided that patient confidentiality is maintained in accord with local requirements.
It will be the monitor's responsibility to inspect the CRFs at regular intervals throughout the study to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them.
The monitor should have access to laboratory test reports and other patient/donor records needed to verify the entries on the CRF.
The investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.
The end of the study corresponds to the date of the last close-out visit in the last center.

11.11 Confidentiality of trial documents and subject records

The investigator must assure that subjects' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents submitted to the sponsor subjects should not be identified by their names but by an identification code and fake initials . The investigator should keep a subject enrolment log showing codes names. The investigator should maintain documents not for submission to MolMed (e.g. written informed consent) in strict confidence.

11.12 Publication of data and protection of trade secrets

The results of this study may be published or presented at scientific meetings. In this case the investigator agrees to discuss all manuscript or abstract with MolMed prior to submission.

This allows the Sponsor to protect proprietary information and to provide comments based on information from other studies that may not be yet available to the investigator.
In accord with standard editorial and ethical practice MolMed will generally support publication of multicentric trials only in their entirety and not as individual centre data. Authorship will be determined by mutual agreement.

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Appendix A: Suggested conditioning regimens

REDUCED TOXICITY CONDITIONING REGIMEN*		
Drug	**Dose**	**Timing**
Treosulfan	14 g/sqm iv	days -6 to -4
Fludarabine	30 mg/sqm/die iv	days -6 to -2
ATG-Thymoglobuline ® ATG-Fresenius	2.5 mg/kg/die OR 10 mg/kg/die iv **	days -5 to -2 days -4 to -2
Rituximab	500 mg iv	day -1
TBI	200 cGy, single fraction	day 0
*For non-US centers only.** If ATG Fresenius® is approved in the country		

TOTAL BODY IRRADIATION (TBI) CONTAINING REGIMEN		
Drug	**Dose**	**Timing**
TBI	7.5 Gy delivered in a single fraction OR 2 Gy twice daily	day -9 days -8, -7, -6
Thiotepa	10 mg/kg iv, divided in two 4 hours infusions (5 mg/kg/q12h)	day -8
Fludarabine	40 mg/sqm/die iv	day -7 > -3
ATG-Thymoglobuline ® 1. ATG-Fresenius	2.5 mg/kg/die OR 10 mg/kg/die iv **	days -5 to -2 days -4 to -2
Rest	-	day –1
** If ATG Fresenius® is approved in the country		

REDUCED INTENSITY CONDITIONING REGIMEN WITHOUT TBI		
Drug	**Dose**	**Timing**
Melphalan	140 mg/sqm iv	days -9
Thiotepa	13 mg/kg iv, divided in two infusions (6.5 mg/kg/q12h)	days -8
Fludarabine	40 mg/sqm/die iv	day -7 > -3
ATG-Thymoglobuline ® 2. ATG-Fresenius	2.5 mg/kg/die OR 10 mg/kg/die iv **	days -5 to -2 days -4 to -2
Rest	-	day –1
** If ATG Fresenius® is approved in the country		

REDUCED-INTENSITY CONDITIONING REGIMEN WITH HIGH-DOSE, POST HCT CYCLOPHOSPHAMIDE		
Drug	**Dose**	**Timing**
Cyclophosphamide	14.5 mg/kg/die	days -6 and -5
Fludarabine	30 mg/sqm/die iv	days -6 to -2
TBI	2 Gy	day –1

MYELOABLATIVE CONDITIONING REGIMEN WITH HIGH-DOSE, POST HCT CYCLOPHOSPHAMIDE		
Drug	**Dose**	**Timing**
Busulfan	110 mg/sqm	days -7 and -4
Cyclophosphamide	14.5 mg/kg/die	days -3 and -2
Fludarabine	25 mg/sqm/die iv	days -6 to -2

Appendix B: Tests for Donor Lymphocytoaphaeresis collection (Arm A)

The following tests have to be performed within 30 days before lymphocytoaphaeresis collection:

Test*
Anti-HCV Antibody (Ab)
HCV RNA (NAT1)
HIV 1-2 p24 AB and Ag
HIV RNA (NAT1)
Anti Treponema Pallidum (Total Ig) - Non specific test *In the case of positive result perform specific test*
Anti Treponema Pallidum - Specific Test (if applicable)
AUSTRALIA HBsAg
Total anti HB core Ab *In the case of positive result perform HBV DNA (NAT1)*
HBV DNA (NAT1)
Anti-Mycoplasma IgM
HTLV I
HTLV II
West Nile Virus**
* according to 2006/17/EC; ** for US sites only

Appendix C: Acute GvHD grading

STAGE	SKIN	LIVER	GASTROINTESTINAL
1	Rash on < 25% of skin*	Bilirubin 2-3 mg/dl**	Diarrhoea > 500 ml/day# or persistent nausea§
2	Rash on 25-50% of skin	Bilirubin 3-6 mg/dl	Diarrhoea > 1000ml/day
3	Rash on > 50% of skin	Bilirubin 6-15 mg/dl	Diarrhoea > 1500 ml
4	Generalized erythroderma with bullous formation	Bilirubin > 15 mg/dl	Severe abdominal pain with or without ileus

* Use "Rule of Nines" or burn chart to determine extent of rash
** Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubin has been documented
# Downgrade one stage if an additional cause of diarrhoea has been documented
§ Persistent nausea with histologic evidence of GvHD in the stomach or duodenum

GRADE	SKIN	LIVER	GASTROINTESTINAL
1	Stage 1-2	none	None
2	Stage 3 or	Stage 1 or	Stage 1
3	-	Stage 2-3 or	Stage 2-4
4†	Stage 4 or	Stage 4	-

† Grade 4 may also include lesser organ involvement but with extreme decrease in performance status

According to:
Przepiorka P, Weisdorf D, Martin P et al, 1994 Consensus Conference on Acute GVHD Grading. BMT, 1995 Jun;15(6):825-8

Appendix D: Chronic GvHD grading

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ KPS ECOG LPS	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthyosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Poikiloderma ☐ Sclerotic features ☐ Pruritus ☐ Hair involvement ☐ Nail involvement **% BSA involved** ☐	☐ No Symptoms	☐ <18% BSA with disease signs but **NO** sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
EYES Mean tear test (mm): ☐ >10 ☐ 6-10 ☐ ≤5 ☐ Not done	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca
GI TRACT	☐ No symptoms	☐ Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss (5-15%)	☐ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
LIVER	☐ Normal LFT	☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN	☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN	☐ Bilirubin or enzymes > 5 x ULN

Figure 1. Organ scoring of chronic GVHD. *AP may be elevated in growing children, and not reflective of liver dysfunction. †Pulmonary scoring should be performed using both the symptom and pulmonary function testing (PFT) scale whenever possible. When discrepancy exists between pulmonary symptom or PFT scores the higher value should be used for final scoring. Scoring using the Lung Function Score (LFS)

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
LUNGS† FEV1 ☐ DLCO ☐	☐ No symptoms ☐ FEV1 > 80% **OR** LFS=2	☐ Mild symptoms (shortness of breath after climbing one flight of steps) ☐ FEV1 60-79% **OR** LFS 3-5	☐ Moderate symptoms (shortness of breath after walking on flat ground) ☐ FEV1 40-59% **OR** LFS 6-9	☐ Severe symptoms (shortness of breath at rest; requiring 0_2) ☐ FEV1 ≤39% **OR** LFS 10-12
JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
GENITAL TRACT	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum

Other indicators, clinical manifestations or complications related to chronic GVHD (check all that apply and assign a score to its severity (0-3) based on its functional impact where applicable (none – 0,mild -1, moderate -2, severe – 3)

Esophageal stricture or web___	Pericardial Effusion___	Pleural Effusion(s)___
Ascites (serositis)___	Nephrotic syndrome___	Peripheral Neuropathy___
M yasthenia Gravis___	Cardiomyopathy___	Eosinophilia > 500µl___
Polymyositis___	Cardiac conduction defects___	Coronary artery involvement___
Platelets <100,000/µl ___	Progressive onset___	

OTHERS: Specify:_____

Figure 1 (continued). is preferred, but if DLCO is not available, grading using FEV1 should be used. The LFS is a global assessment of lung function after the diagnosis of bronchiolitis obliterans has already been established [29]. The percent predicted FEV1 and DLCO (adjusted for hematocrit but not alveolar volume) should be converted to a numeric score as follows: >80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; <40% = 6. The LFS = FEV1 score + DLCO score, with a possible range of 2-12. GVHD indicates graft versus host disease; ECOG, Eastern Cooperative Oncology Group; KPS, Karnofsky Performance Status; LPS, Lansky Performance Status; BSA, body surface area; ADL, activities of daily living; LFTs, liver function tests; AP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal.

Grade	Specification	Score
Mild	Involves 1 or 2 organs or sites (exception the lung see below), with no clinically significant functional impairment	1 in all affected organs or sites
Moderate	Involves at least 1 organ or site with clinically significant but no major disability	2 in any affected organ or site
	Involves ≥3 organs or sites with no clinically significant functional impairment	1 in all affected organs or sites
	lung	1
Severe	Major disability	3 in any organ or site
	lung	≥ 2

According to:
Filipovich AH, Weisdorf D, Pavletic S et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005 Dec;11(12):945-56.

Appendix E: ECOG performance status scale

Grade	Performance scale
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.: Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

Appendix F: Optional Functional studies

The following optional additional phenotyping panels regarding the functional characterization of immune reconstitution will be performed locally by participating sites at 15 days, 6 months and 1 year after IR**:**

CD45/CD3/CD4/CD8/CD27/CD28
CD45/CD3/CD4/CD45RA/CD62L/CD31/CD127
CD45/CD3/CD4/CD25/HLA-DR

For Arm A only:
CD45/LNGFR/CD4/CD8/CD27/CD28
CD45/LNGFR/CD4/CD45RA/CD62L/CD31/CD127
CD45/LNGFR/CD4/CD25/HLA-DR

After staining, the red cells in each tube will be lysed and the remaining cells may be fixed with paraformaldehyde. During analysis in the flow cytometer, initial gating will be performed to include all events bearing CD45. Lymphocytes will be further identified and electronically gated on forward and orthogonal light scatter signals. The fluorescent signals for phenotype analyses will be accumulated for the gated lymphocytes. Events representing cells binding the relevant markers will be identified by their light scatter and fluorescence signatures. The internal standard of microbeads shall be used to calculate the absolute numbers of pertinent cells in each assay tube. The instrument raw data shall be stored electronically for archiving, data processing, and reporting.

Immune competence analyses
Functional studies aimed to characterize the pattern of immune-reconstitution will be performed centrally at MolMed laboratory:

- Before conditioning regimen
- Before the first TK cell infusion (applicable for patients enrolled in arm A)
- Fifteen days after IR
- 6 months after IR
- 1 year after IR

The following analyses will be performed:

1. Analysis of cytokine produced by lymphocytes

This assay is performed by intracytoplasmic staining with PE or FITC-conjugated antibodies directed to γIFN, IL-2, IL-4 after 6 hours exposure to polyclonal mitogens, and block of cytokine secretion by exposure to Brefeldin. Cells are stained also with anti-CD3 antibodies and results are analysed on CD3+ cells. Cells from healthy donors are utilized as controls.

2. IFNγ production to cells expressing viral antigens such as CMV, EBV

This assay is performed by ELISPOT for γIFN. Briefly, circulating lymphocytes from treated patients will be challenged with: 1) Anti-CD3+anti-CD28 (positive controls); 2) Immunodominant peptides from viral antigens (samples); 3) Medium (negative control).

3. Molecular analysis for rearrangement of the T cell receptor. This assay will be performed by spectratyping.

Analysis of the cytokine pattern produced by transduced lymphocytes
This assay is performed by intracytoplasmic staining with PE or FITC-conjugated antibodies directed to γIFN, IL-2, IL-4, after 6 hours exposure to polyclonal mitogens, and block of cytokine secretion by exposure to Brefeldin. Cells are stained also with anti-LNGFR antibodies and results are analysed on LNGFR+ cells. Cells from healthy donors are utilized as controls.

4. Detection of antigen-specific transduced T cells by the use of tetramers/pentamers

This analysis can be performed on those patients with donor or host HLA typing matched to the available tetramers or pentamers, commercially available, designed to detect T cells specific CMV and EBV-related antigens. The analysis is performed by staining with antibodies conjugated with fluorochromes, specific for CD3, LNGFR, CD8 and a tetramer or pentamer of HLA- molecules bound to a CMV or EBV immunodominant peptides restricted. This analysis allows the quantification of T cells specific for antigens relevant in the transplant setting within the total population of circulating T lymphocytes and circulating transduced cells.

For Arm A, at the first determination of circulating LNGFR+ cells > 50/µl, 50 ml of blood will be harvested. Cells will be processed and stocked at each center site (if possible), to allow potential future determination of safety profile.

Immunological follow-up for selected clinical conditions
In case of unexpected disappearance of transduced donor lymphocytes from circulation, an immune response to the transgene will be investigated.
In the presence of GvHD, PBMC are harvested before ganciclovir treatment, 4 days after the beginning of ganciclovir or valganciclovir treatment and 1 day after the discontinuation of ganciclovir or valganciclovir. Analysis of host-reactivity will be performed by 72 hours by ELISPOT for γIFN.

Sample collection procedures
Each sample will be collected into vacutainer tube bearing an appropriate label. 10 ml blood sample should allow to collect at least 5 x 10^6 PBMC/cryotube whereas the 50 ml blood sample should allow to collect approximately 20 x 10^6 PBMC/vials slip into two vials.
Each sample of collected PBMC will be harvested, processed (by density gradient centrifugation) and frozen as viable cells. MolMed will arrange the shipment of the stored samples from the clinical site to central laboratory in MolMed.

Appendix G: Common terminology criteria for Adverse Events

In the present study adverse events and/or adverse drug reactions will be recorded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.02.
At the time this protocol was issued, the full CTC document was available on the NCI web site, at the following address:
http://ctep.cancer.gov/reporting/ctc.html

Appendix H: Glossary

AE	Adverse Event
AL	Acute Leukemia
ALL	Acute Lymphoblastic Leukemia
ALT	Alanine Amino Transferase
AML	Acute Myeloid Leukemia
AST	Aspartate Transaminase
ATG	Anti-Thymocyte Globuline
BM	Bone Marrow
BSE	CMV
BUN	Blood Urea Nitrogen
CAR	Chimeric Antigen Receptor
CML	Chronic Myeloid Leukemia
CMV	Cytomegalovirus
CNS	Central Nervous System
CY	Cyclophosphamide
DLCO	Diffusing Lung Capacity of Carbon Oxide
DLI	Donor Lymphocyte Infusion
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr Virus
EFS	Event Free Survival
EVA	Ethylene Vinyl Acetate
FDA	Food and Drug Administration
G-CSF	Granulocyte colony-stimulating factor
GCV	Ganciclovir
γGT	Gamma-glutamyl transpeptidase
GMP	Good Manufacturing Practice
GvHD	Graft versus Host Disease
GvL	Graft versus Leukemia
Hb	Haemoglobin
HCT	Hematopoietic Cell Transplantation
HD	Hodgkin Disease
HLA	Human Leukocyte Antigen
HSA	Human Serum Albumin
HSC	Hematopoietic Stem Cell
HCT	Hematopoietic Stem Cell Transplantation

HSV-Tk	Herpes Simplex Virus Thymidine Kinase
Hct	Haematocrit
IFNγ	Interferon Gamma
IL-2	Interleukin 2
IL-4	Interleukin 4
IR	Immune reconstitution
ITT	Intention To Treat
IU	International Units
LDH	Lactate Dehydrogenase
LFS	Leukemia Free Survival
LNGFR	Low Affinity Nerve Growth Factor Receptor
MCV	Mean Corpuscolar Volume
MDS	Myelodysplastic Syndrome
MPV	Mean Platelet Volume
MUGA	Multigated acquisition
NGF	Nerve Growth Factor
NHL	Non Hodgkin's Lymphoma
NIH	National Institutes of Health
NK	Natural Killer
NRM	Non Relapse Mortality
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
RAEB-T	Refractory anemia with excess blasts
RCR	Replication Competent Retrovirus
RNA	Ribonucleic acid
SADR	Seriuos Adverse Drug Reaction
SAE	Serious Adverse Event
SCT	Stem Cell Transplantation
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBI	Total body irradiation
TCR	T-Cell Receptor
TRM	Transplant Related Mortality

Appendix I: Creatinine clearance formula

Evaluation of estimated creatinine clearance according to Cockcroft-Gault formula

If serum creatinine is expressed in mg/dl

FOR MALES Creatinine clearance = $\frac{(140 - \text{age [years]}) \times (\text{body weight [Kg]})}{(\text{serum creatinina [mg/dl]}) \times 72}$

FOR FEMALES $\text{Creatinine clearance}_{\text{woman}}$ = 0.85 x male value

If serum creatinine is expressed in µmol/l

FOR MALES Creatinine clearance = $\frac{(140 - \text{age[years]}) \times (\text{body weight [Kg]})}{(\text{serum creatinina [µmol/l]}) \times 0.81}$

FOR FEMALES $\text{Creatinine clearance}_{\text{woman}}$ = 0.85 x male value

Appendix J: Quality of life assessment

FACT-BMT (Version 4)

Below is a list of statements that other people with your illness have said are important. **By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.**

	PHYSICAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please check this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

3/20/03
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FACT-BMT (Version 4)

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

FACT-BMT (Version 4)

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
BMT1	I am concerned about keeping my job (include work at home)	0	1	2	3	4
BMT2	I feel distant from other people	0	1	2	3	4
BMT3	I worry that the transplant will not work	0	1	2	3	4
BMT4	The effects of treatment are worse than I had imagined	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
C7	I like the appearance of my body	0	1	2	3	4
BMT5	I am able to get around by myself	0	1	2	3	4
BMT6	I get tired easily	0	1	2	3	4
BL4	I am interested in sex	0	1	2	3	4
BMT7	I have concerns about my ability to have children	0	1	2	3	4
BMT8	I have confidence in my nurse(s)	0	1	2	3	4
BMT9	I regret having the bone marrow transplant	0	1	2	3	4
BMT 10	I can remember things	0	1	2	3	4
Br1	I am able to concentrate (e.g., reading)	0	1	2	3	4
BMT 11	I have frequent colds/infections	0	1	2	3	4
BMT 12	My eyesight is blurry	0	1	2	3	4
BMT 13	I am bothered by a change in the way food tastes	0	1	2	3	4
BMT 14	I have tremors	0	1	2	3	4
B1	I have been short of breath	0	1	2	3	4
BMT 15	I am bothered by skin problems (e.g., rash, itching)	0	1	2	3	4
BMT 16	I have trouble with my bowels	0	1	2	3	4
BMT 17	My illness is a personal hardship for my close family members	0	1	2	3	4
BMT 18	The cost of my treatment is a burden on me or my family	0	1	2	3	4

Appendix K: Long term follow up questionnaire (LTFUQ)

Patient code |_|_|_|_|_|_|_|

Patient's initials |_|_|_|_|

Date of birth |_|_|_|_|_|_| D D M M Y Y

Date of visit |_|_|_|_|_|_| D D M M Y Y

Since the last annual physical exam and/or questionnaire, does the patient develop any of the following potential risks?

Please add additional pages if necessary

1. Immune system (e.g. infections)

☐ NO ☐ YES

If Yes, please specify, including onset date, duration, severity and outcome of event(s):

Onset date |__|__|__|__|__|__|__|__| D D M M M Y Y

Duration |____| ☐ Days ☐ Weeks ☐ Months

Severity
☐ mild
☐ moderate
☐ severe
☐ life-threatening
☐ death

Outcome: ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown

2. Ocular (e.g. cataracts, sicca syndrome, microvascular retinopathy)

☐ NO ☐ YES

If Yes, please specify, including onset date, duration, severity and outcome of event(s):

Onset date |__|__|__|__|__|__|__|__| D D M M M Y Y

Duration |____| ☐ Days ☐ Weeks ☐ Months

Severity
☐ mild
☐ moderate
☐ severe
☐ life-threatening
☐ death

Outcome: ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown

3. Oral (e.g. caries, sicca syndrome)

☐ NO ☐ YES	**If Yes,** please specify, including onset date, duration, severity and outcome of event(s): **Onset date** **Duration** **Severity** |__|__|__|__|__|__|__| |____| ☐ Days ☐ Weeks ☐ Months ☐ mild ☐ moderate ☐ severe ☐ life-threatening ☐ death D D M M M Y Y **Outcome:** ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown
4. Respiratory (e.g. idiopathic pneumonia syndrome, bronchiolitis obliterans syndrome, cryptogenic organizing pneumonia, sinopulmonary infections)	
☐ NO ☐ YES	**If Yes,** please specify, including onset date, duration, severity and outcome of event(s): **Onset date** **Duration** **Severity** |__|__|__|__|__|__|__| |____| ☐ Days ☐ Weeks ☐ Months ☐ mild ☐ moderate ☐ severe ☐ life-threatening ☐ death D D M M M Y Y **Outcome:** ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown
5. Cardiac & vascular (e.g. cardiomyopathy, congestive heart failure, arrhythmias, valvular anomaly, coronary artery disease, cerebrovascular disease, peripheral arterial disease)	
☐ NO ☐ YES	**If Yes,** please specify, including onset date, duration, severity and outcome of event(s): **Onset date** **Duration** **Severity** |__|__|__|__|__|__|__| |____| ☐ Days ☐ Weeks ☐ Months ☐ mild ☐ moderate ☐ severe ☐ life-threatening ☐ death D D M M M Y Y **Outcome:** ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown
6. Liver (e.g. GvHD, HBV, HCV, iron overload)	
☐ NO ☐ YES	**If Yes,** please specify, including onset date, duration, severity and outcome of event(s): **Onset date** **Duration** **Severity** |__|__|__|__|__|__|__| |____| ☐ Days ☐ Weeks ☐ Months ☐ mild ☐ moderate ☐ severe ☐ life-threatening ☐ death D D M M M Y Y **Outcome:** ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown

7. Renal & genitourinary (e.g. chronic kidney disease, bladder dysfunction, urinary tract infections)

☐ NO ☐ YES

If Yes, please specify, including onset date, duration, severity and outcome of event(s):

Onset date |__|__|__|__|__|__|__|__| D D M M M Y Y

Duration |____| ☐ Days ☐ Weeks ☐ Months

Severity
☐ mild
☐ moderate
☐ severe
☐ life-threatening
☐ death

Outcome: ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown

8. Muscle & connective tissue (e.g. myopathy, fascitis/scleroderma, polymyositis)

☐ NO ☐ YES

If Yes, please specify, including onset date, duration, severity and outcome of event(s):

Onset date |__|__|__|__|__|__|__|__| D D M M M Y Y

Duration |____| ☐ Days ☐ Weeks ☐ Months

Severity
☐ mild
☐ moderate
☐ severe
☐ life-threatening
☐ death

Outcome: ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown

9. Skeletal (e.g. osteopenia/osteoporosis, avascular necrosis)

☐ NO ☐ YES

If Yes, please specify, including onset date, duration, severity and outcome of event(s):

Onset date |__|__|__|__|__|__|__|__| D D M M M Y Y

Duration |____| ☐ Days ☐ Weeks ☐ Months

Severity
☐ mild
☐ moderate
☐ severe
☐ life-threatening
☐ death

Outcome: ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown

10. Nervous system (e.g. leukoencephalopathy, late infections, neuropsychological & cognitive deficits, calcineurin neurotoxicity, peripheral neuropathy)

☐ NO ☐ YES

If Yes, please specify, including onset date, duration, severity and outcome of event(s):

Onset date |__|__|__|__|__|__|__|__| D D M M M Y Y

Duration |____| ☐ Days ☐ Weeks ☐ Months

Severity
☐ mild
☐ moderate
☐ severe
☐ life-threatening
☐ death

Outcome: ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown

11. Endocrine (e.g. hypothyroidism, hypoadrenalism, hypogonadism, growth, retardation)	
☐ NO ☐ YES	**If Yes,** please specify, including onset date, duration, severity and outcome of event(s): **Onset date** **Duration** **Severity** |__|__|__|__|__|__|__|__| |____| ☐ Days ☐ Weeks ☐ Months ☐ mild ☐ moderate ☐ severe ☐ life-threatening ☐ death D D M M M Y Y **Outcome:** ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown
12. Mucocutaneous (e.g. cutaneous sclerosis, genital GvHD)	
☐ NO ☐ YES	**If Yes,** please specify, including onset date, duration, severity and outcome of event(s): **Onset date** **Duration** **Severity** |__|__|__|__|__|__|__|__| |____| ☐ Days ☐ Weeks ☐ Months ☐ mild ☐ moderate ☐ severe ☐ life-threatening ☐ death D D M M M Y Y **Outcome:** ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown
13. Second cancer (e.g. solid tumors, hematologic malignancies, PTLD)	
☐ NO ☐ YES	**If Yes,** please specify, including onset date, duration, severity and outcome of event(s): **Onset date** **Duration** **Severity** |__|__|__|__|__|__|__|__| |____| ☐ Days ☐ Weeks ☐ Months ☐ mild ☐ moderate ☐ severe ☐ life-threatening ☐ death D D M M M Y Y **Outcome:** ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown

14. Psychosocial & sexual (e.g. depression, anxiety, fatigue, sexual dysfunction)

☐ NO ☐ YES

If Yes, please specify, including onset date, duration, severity and outcome of event(s):

Onset date **Duration** **Severity**

|__|__|__|__|__|__|__|__| |____| ☐ Days ☐ Weeks ☐ Months

D D M M M Y Y

☐ mild
☐ moderate
☐ severe
☐ life-threatening
☐ death

Outcome: ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown

15. Fertility (infertility)

☐ NO ☐ YES

If Yes, please specify, including onset date, duration, severity and outcome of event(s):

Onset date **Duration** **Severity**

|__|__|__|__|__|__|__|__| |____| ☐ Days ☐ Weeks ☐ Months

D D M M M Y Y

☐ mild
☐ moderate
☐ severe
☐ life-threatening
☐ death

Outcome: ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown

16. General health

☐ NO ☐ YES

If Yes, please specify, including onset date, duration, severity and outcome of event(s):

Onset date **Duration** **Severity**

|__|__|__|__|__|__|__|__| |____| ☐ Days ☐ Weeks ☐ Months

D D M M M Y Y

☐ mild
☐ moderate
☐ severe
☐ life-threatening
☐ death

Outcome: ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown

17.1. Events of special interest: Graft versus Host Disease	
☐ NO ☐ YES	**If Yes,** please specify, including onset date, duration, severity and outcome of event(s): **Onset date** **Duration** **Severity** |__|__|__|__|__|__|__| |____| ☐ Days ☐ Weeks ☐ Months ☐ mild ☐ moderate ☐ severe ☐ life-threatening ☐ death D D M M M Y Y **Outcome:** ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown
17.2. Events of special interest **Development of Immunological events, e.g. autoimmunity, allergic reaction, antibody formation,** **_____** **(please specify)**	
☐ NO ☐ YES	**If Yes,** please specify, including onset date, duration, severity and outcome of event(s): **Onset date** **Duration** **Severity** |__|__|__|__|__|__|__| |____| ☐ Days ☐ Weeks ☐ Months ☐ mild ☐ moderate ☐ severe ☐ life-threatening ☐ death D D M M M Y Y **Outcome:** ☐ Fatal ☐ Resolved ☐ Resolved with sequelae ☐ Improved ☐ Persistent ☐ Worsened ☐ Unknown
General remarks	

Hospital address			
Physician name			
Signature		**Date**	|__|__|__|__|__|__| D D M M Y Y

Appendix L: Amendment history

Protocol Version	Protocol Approval Date	Substantial Changes
IPR/21.B	21/02/2011	• It has been changed the primary objective of the study, from non-relapse mortality (NMR) to disease-free survival (reference § 2 and 2.1) • It has been modified the sample size, from 152 to 170 patients (reference § 11.4). • It has been included the comorbidity index, the definition of high risk of relapse and liver, cardiac, kidney and pulmonary parameters in the inclusion criteria (reference § 4.2, appendix H and I). • It has been defined the use of ATG Fresenius only if approved in the Country (reference § 4.5.1). • It has been included the monitoring of vital signs during the infusion of HSV-Tk cells (reference §5.4). • It has been defined the donor's test (reference §6.1.2) • It has been specified the procedure for AE&SAE collection (reference §6.4.3) • It has been included a sample collection at baseline for functional studies (reference §8.2)
IPR/21.C	28/07/2011	• It has been specified the donor's tests to perform according to EU and US regulations (reference 6.1.2) • It has been included the molecular analysis for rearrangement of the T cell receptor in the immune competence analyses and during the long term follow-up (reference §8.2 and 13.5.4)
IPR/21.D	13/06/2012	• It has been added in the inclusion criteria patients with AML and ALL in 1st or 2nd relapse or primary refractory (reference §4.2) • It has been included in the Control Arm B the investigator's choice to perform the unmanipulated haploidentical bone marrow transplantation followed by high-dose of cyclophosphamide on days +3 and +4. (Reference §6.1 and 6.2.2).
IPR/21.E	18/02/2016	• The comparison of time to GvHD resolution and use of immunosuppressive agents has been added among the secondary study aims for specific request of the European Medicines Agency (reference §2.2). • Study results of the phase I/II study TK007 have been updated (reference §1.3.2).
IPR/21.F	23/06/2017	• The comparison of GRFS has been added among the secondary study aims (reference §2.2). • TK008 trial has been classified as a category 2 study, as a part of Conditional Marketing Authorization of the medicinal product Zalmoxis (Reference §3). • The comorbidity index and the term "high risk of relapse" have been eliminated from the inclusion criteria #1 and 2.1, respectively, because already foreseen by the exclusion criteria #2 "contraindication to haploidentical HCT" (Reference §4.2). • The inclusion criteria # 3 has been better defined (Reference §4.2).

		• The wash-out period of 24 hours after G-CSF and immunosuppressive therapy administration has been further defined (Reference §5.5). • The recommended conditioning regimens have been reported as Appendix A • It has been specified that the supportive care can be administered according to institutional clinical practice (Reference §4.5). • Procedures of drug administration have been further detailed (Reference §5.4). • Donor tests for lymphocytoapheresis collection have been listed as Appendix B • The HLA testing for patients of Arm A has been deleted • Disease assessments and the follow-up after disease relapse or progression have been better defined (Reference §6.3.1). • Survival follow up and procedures of safety collection have been better defined (Reference §6.3.5, 6.3.7, 10.3.1 and 10.4). • Analyses regarding functional characterization of immune reconstitution have been defined as optional studies and reported in Appendix F • Considering that as part of the CMA granted for the medicinal product, EMA will review the safety data of this study on an every-6-month basis through the PSURs and the benefits and risks of the medicinal product on an annual basis, the Data and Safety Monitoring Board will no longer be operational. The risk management plan has been updated (Reference § 10.5) • The long term follow-up has been further defined and the test of rearrangement of TCR repertoire has been eliminated, as it is considered no longer appropriate (Reference §10.5.4) • The use of the alert card for treated patients has been included (Reference §10.5.4) • The section 11 of ethics and general study administration on guidance, traceability records, informed consent and electronic CRF has been updated.