



A phase I-II study: infusion of donor lymphocytes transduced with the suicide gene HSV TK, after transplantation of allogeneic T-depleted stem cells from a haploidentical donor in patients with haematological malignancies

Compound: HSV-TK

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| MolMed S.p.A. | CLINICAL STUDY PROTOCOL | Internal Code: IPR/01 G |
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Synopsis of the Protocol

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| Title | A phase I-II study: infusion of donor lymphocytes transduced with the suicide gene HSV-tk after transplantation of allogeneic T-depleted stem cell from a related donor with partial compatibility (haploidentical donor) in patients with haematological malignancies |
| Sponsor | MolMed S.p.A. |
| Indication | Improvement of immune reconstitution in patients undergoing haploidentical SCT |
| Objectives | The aim of the study is to obtain immune reconstitution as well as reduction of infective episodes and disease relapse in patients with haematological malignancies who underwent SCT (and subsequent T lymphocytes infusions) and selectively controlling GvHD |
| Study design | Phase I-II trial, multicentric open and non randomised |
| Number of patients | 30 evaluable |
| Study population | Patients \geq 18 years of age affected by haematological malignancies who underwent T depleted haploidentical SCT |
| Study procedures | <p>Donor lymphocytoapheresis, transduction and selection of the lymphocytes</p> <p>Infusion of 1×10^7 CD3+ c/kg (or alternatively 1×10^6 CD3+ c/kg at Investigator discretion based on estimated infectious risk) genetically modified between day +21 and day +49 after SCT</p> <p>In absence of immune reconstitution and GvHD further infusions will be administered with the following dosages and timelines:</p> <p>30 days after 1st infusion: 1×10^7 CD3+ c/kg</p> <p>30 days after 2nd infusion: 1×10^6 CD3+ c/kg + IL-2 (6.000.000 IU/m² sc x 5 days)</p> <p>30 days after 3rd infusion: 1×10^7 CD3+ c/kg + IL-2 (6.000.000 IU/m² sc x 5 days)</p> <p>In case of patients transplanted in relapse and/or in relapse after transplant, the participating clinical center can apply for permission to start treatment with a different dose (increased) from the first scheduled in the protocol. In any case, this should be approved by the sponsor through submission of appropriate documentation (CRFs) on disease status at screening and or pre infusion and appropriate rationale for such modification of the treatment schedule.</p> <p>In case of GvHD \geq grade 2, ganciclovir will be administered at dosage of 10 mg/kg/day in 2 administrations for 14 days.</p> <p>Steroids and Cyclosporine A will be administered in case of uncontrolled GvHD</p> |

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Synopsis of the Protocol

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| Duration | Duration of the trial will be related to the outcome during the infusional phase (presence or not of immune reconstitution and GvHD). Indicatively, the infusional phase will lasts up to 5 months from the SCT and the follow up will lasts 6 month. In presence of relapse patient can be treated with DLI also during the post follow up phase |
| Product | HSV-tk |
| Comparator | Not applicable |
| Efficacy | The efficacy of the transduction system and the transgene will be evaluated on the basis of GvHD control and induction of immune reconstitution |
| Safety and tolerability | Adverse events, Serious Adverse Events and modification during the trial of clinical and lab parameters from screening |
| Pharmacokinetics | Not applicable |
| Pharmacodinamics | Not applicable |
| Quality of life | Not applicable |
| Statistical analysis | Frequency tables for safety and activity |

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1 Introduction

1.1 Background and rationale

The aim of the proposed trial is to improve immunological reconstitution and consequently reduce infectious episodes while preventing disease relapse in patients affected by haematological malignancies who have undergone stem cell transplantation (SCT) and infusion of genetically modified T lymphocytes obtained from a haploidentical donor.

The potential onset of Graft versus Host Disease (GvHD) will be selectively controlled by the transduction of donor lymphocytes with a retroviral vector containing a suicide gene.

After allogeneic SCT, the infusion of donor lymphocytes induces the reconstitution of antiviral immunity and mediates a curative immune response against the haematological disease; however these lymphocytes may also cause GvHD. This severe limitation significantly reduces the efficacy of donor lymphocytes, especially in the context of haploidentical transplant.

The proposed trial consists of administration of donor T lymphocytes genetically modified to express the suicide thymidine kinase gene from Herpes Simplex Virus (HSV-tk). This gene makes the transduced cells sensitive to ganciclovir. In the event of GvHD, the administration of ganciclovir will eliminate in a selective way the previously infused donor T cells, thereby allowing for its control.

1.2 Use of donor lymphocytes after transplantation of hematopoietic stem cells

Allogeneic bone marrow transplantation is the therapy of choice for a number of hematopoietic malignancies such as leukaemia, lymphoma and multiple myelomas^{1,2}.

The immunological role of donor T lymphocytes in the eradication of the tumours is universally recognized. The added antitumoral effect derived from the donor T lymphocytes (Graft versus Leukemia, GvL) makes this therapeutic method the preferential choice for patients who have a consanguineous or marrow bank donor. Donor lymphocytes infusions (DLI) have been shown to be effective in the treatment of a large number of post-transplantation relapses, with a success rate of between 15 to 80 % depending on the type of disease. The best responses are obtained in the treatment of chronic myelogenous leukaemia, myeloma, lymphoma and to a lesser degree, in acute leukemias³⁻¹³. 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 transplant¹⁴⁻¹⁹.

However, the infusion of donor T lymphocytes increases the risk of GvHD^{8,12,13} while 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.

1.3 Strategy to control GvHD while increasing both GvL and immune reconstitution

There is a strong association between GvL and GvHD suggesting a common biological mechanism. However, the specific contribution of the different lymphocyte subpopulations remains unclear²⁰⁻²⁴. 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.

The use of escalating doses of donor lymphocytes (escalating dose regimen, EDR) provides an antitumor efficacy equal to that of protocols using high dose single infusion of lymphocytes (bulk dose regimen, BDR), while significantly reducing the incidence of GvHD^{25,26}. An alternative strategy is to infuse antigen-specific lymphocytes.

Although the targets for the anti-leukemic response are still not known, it is possible to isolate donor lymphocytes clones specific for minor histocompatibility antigens with restricted tissue distribution. Some of these antigens could be selectively expressed on the patient hematopoietic cells and leukemic blasts²²⁻²⁷. The infusion of donor lymphocytes specific for such antigens could induce a selective GvL effect in the absence of GvHD. This hypothesis, however, has not yet found clinical evidence. At present, most patients receive a polyclonal population of lymphocytes, increasing the risk of GvHD, which is particularly high in patients who undergo SCT from a haploidentical donor.

1.4 Bone marrow transplantation from a related haploidentical donor

At present, less than 30% of the patients who could benefit from an allogeneic transplant have an HLA identical family donor and only 30% are able to find a compatible unrelated donor²⁸. The search for an unrelated donor could last a minimum of 3-4 months and for patients who do not achieve durable remission with standard therapies or who relapse early and lack a compatible donor, the best chance of an allogeneic bone marrow transplantation is from a related donor who is only partially compatible (haploidentical donor).

Studies in animal models have demonstrated that it is possible to overcome the HLA barrier and avoid rejection of the haploidentical hematopoietic cells by increasing the dose of stem cells^{29,30}. In the clinical environment, similar results could be obtained by increasing the dose of stem cells in the graft and using hematopoietic progenitor cells (PBPC) mobilized with a growth factor. The donation of PBPC has similar risks to the donor as bone marrow donation^{31,32}. In haploidentical unmanipulated transplants, the related mortality caused principally by rejection or GvHD, ranges between 50 and 70%^{33,34}.

The *in vitro* depletion of T lymphocytes, is one of the most efficient methods for the prevention of acute GvHD³⁵.

This method has now been applied to transplantation from haploidentical donors. Aversa and colleagues have demonstrated that high doses of PBPC, with very low doses of T cells and intensive immunosuppressive effect in the conditioning, allow for a rapid, complete and stable engraftment of haploidentical PBPC in the absence of GvHD^{36,37}.

Unfortunately, the depletion of T cells results in immunodeficiency that lasts for more than 1 year after transplantation, even in the absence of immunosuppressive drugs. During this long phase of immunodeficiency, the patients require strict medical care and prophylaxis to control bacterial, fungal and viral infections. Even with these precautions, infections that occur during the first 12 months post transplantation represent the principal cause of death in these patients. Furthermore, experience from HLA matched allogeneic transplantations show that the benefits of T-depletion could be outweighed by the increased risk of rejection and disease relapse (in particular, in chronic myelogenous leukaemia) and of EBV related lymphomas³⁷⁻⁴⁰.

There is a need to develop new strategies to induce rapid immune reconstitution in patients who undergo bone marrow transplantation from a haploidentical donor.

1.5 Rationale for the insertion of the hsv-tk suicide gene in the donor t-lymphocytes

Delayed immune reconstitution remains one of the main problems of haploidentical stem cell transplantation. 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 contributing causes^{36,41}. Attempts to overcome this problem with infusions of donor lymphocytes have been associated with a high incidence of GvHD⁴².

The infusion of transduced lymphocytes should allow for early immunological reconstitution and reduce the risk of infections and disease relapse. The presence of a marker gene will also make it possible to monitor the survival, expansion and site of migration of the genetically modified cells. The proposed suicide gene encodes for the thymidine kinase gene from Herpes Simplex Virus I (HSV-tk) that, once inserted, will render genetically modified cells sensitivity to antiviral drug, ganciclovir.

If GvHD occurs, the donor lymphocytes can be eliminated by the administration of ganciclovir, thus allowing GvHD remission.

Recent clinical studies conducted in European and American centers, have demonstrated the safety and clinical efficacy of the suicide gene HSV-tk insertion in donor lymphocytes^{43,44}.

Results of these studies suggest that the genetically manipulated cells are well tolerated and can survive long term (>4 years) at a high frequency (36% of circulating mononuclear cells). The antitumor and antiviral activity of genetically modified lymphocytes has been proven in nearly 50% of treated patients and the suicide strategy has been successful in all patient who received ganciclovir. It has always been possible to eliminate the transduced cells either partially or entirely with the administration of ganciclovir, achieving a complete remission of all the symptoms and signs of acute GvHD and a partial clinical response in a case of chronic GvHD.

The proposed clinical trial represents an evolution of the data already published that have provided important results in terms of safety and efficacy⁴⁴⁻⁴⁸.

This phase I-II study aims at demonstrating the activity of this therapeutic strategy.

1.6 Description of the suicide strategy based on HSV-tk ganciclovir

Patients who undergo SCT from a haploidentical donor will receive donor lymphocytes that have been genetically modified with the retroviral vector SFCM-3. This vector encodes for both the suicide gene HSV-tk and the marker gene Δ LNGFR.

HSV-tk encodes, as has already been mentioned, the thymidine kinase enzyme of Herpes Simplex Virus I and once inserted into donor lymphocytes, makes them sensitive to ganciclovir. After the administration to the patients, ganciclovir is phosphorylated by HSV thymidine kinase enzyme expressed by the genetically modified cells and then by cellular kinases.

The active form of ganciclovir inhibits the synthesis of genomic DNA, thereby causing cell death^{43,49-51}. The gene Δ LNGFR encodes a low affinity receptor for nerve growth factor (NGF), which has been deleted of its intracellular portion in order to be no longer able to transmit signals⁵².

Using monoclonal antibodies conjugated to magnetic beads, the presence of Δ LNGFR protein allows for the immune selection of genetically modified cells. Furthermore, the expression of the Δ LNGFR protein is used as a marker for genetically modified cells once infused into the patients and allows the demonstration of their presence, possible expansion or reduction and characterization in terms of lymphocyte subtype and state of activation.

2 Description of the gene construct and the methods of obtaining the genetically modified product

The retroviral vector SFCMM-3 has been obtained using the classical techniques of genetic engineering. The original vector is called LXSN⁵³ (Genebank accession #28248), and has been modified by removal of the gene encoding for neomycin resistance (neo) and insertion of the Δ LNGFR and HSV-tk genes, thereby generating the vector SFCMM-3

HSV-I-tk is the gene that encodes the thymidine kinase enzyme of Herpes Simplex Virus I⁵⁴. The derived protein is functional and used both in vitro and in vivo to selectively eliminate transduced cells in the presence of ganciclovir. The transcription of the HSV-tk gene is under the control of the LTR promoter of MoMuLV.

Δ LNGFR is the gene that encodes the low-affinity receptor of Nerve Growth Factor which has its intracellular portion truncated^{52,55,56}.

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 Δ LNGFR using FACS analysis with a specific antibody. Transcription of the Δ LNGFR gene is under the control of the Sv40 early promoter.

The construct obtained has been used to transfect the ecotropic GP+E86 packaging cells line (ATCC n° CRL-9642). The supernatant from the transient transfection was harvested after 48 hours and used to infect the packaging amphotropic GP+env Am12 packaging cell line (available from ATCC n° CRL 9641) from now on referred to as Am12.

The insertion of the construct in these packaging cell lines allows for the production of retroviral vectors with amphotropic spectrum, able to infect mammalian cells, including man. The produced cell line was selected on the basis of expression of Δ LNGFR using specific antibodies conjugated to magnetic beads.

The resulting cell population was plated in limited dilution in order to obtain single producers cell clones. After nearly 15-day culture, the single clones were harvested and expanded.

The clones were tested for expression of Δ LNGFR, sensitivity to ganciclovir, good growth capacity in culture, efficiency of transduction of T lymphocytes, stability, absence of contaminating adventitious viruses and of replication competent forms, as described elsewhere. For the present study clone #35 will be used⁵⁷.

2.1 Cellular banks

Production and storage of cell banks of SFCMM-3 #35 cell line have been performed in MolMed S.p.A. laboratories. All reagents and appropriate cell culture media are quality controlled and composition certified, especially as far as sterility and absence of both mycoplasma and endotoxin are concerned.

Furthermore, the foetal bovine serum necessary for the culture of this cell line is certified for its origin, i.e. deriving from regions free of bovine encephalitis.

Original producer cells were expanded to produce a small cell bank, which was certified free of bacteria, fungi, and mycoplasma. Thereafter, a master cell bank (MCB) was established, and then quality controlled as far as sterility, absence of mycoplasma, of recombinant replication-competent retroviruses (RCR), and of adventitious viruses are concerned.

Finally, identity and stability of the cell line were evaluated analysing, at varying times, the integration process, the number of vector integration sites, and the expression of the genes of interest.

2.2 Retroviral Supernatant

Production of the supernatant containing the viral particles of interest is obtained starting from the WCB. The supernatant collection is performed using serum-free media. The supernatant containing the viral particles of interest is therefore quality controlled as far as sterility, transduction efficiency in the reference CEM A3.01 cell line, vector integrity, absence of mycoplasma, of RCR, and of adventitious viruses are concerned. Finally, sterility and absence of RCR are also verified in producer cells at the end of the process.

2.3 Genetically Modified Lymphocytes

The product to be infused is constituted by engineered T-lymphocytes modified with SFCMM-3 retroviral vector containing HSV-tk and Δ LNGFR genes.

Donor T-cells manipulation will be performed at MolMed S.p.A. via Olgettina 58 Milan and at GMP facilities present at other Institutions participating in the trial.

Appropriate reagents and media for cell culture will be used, certified for composition and quality (in particular sterility, absence of mycoplasma and endotoxin) and with a specified expiry date.

Autologous plasma will be used as a supplement for culture media.

Lymphocytoapheresis will be obtained from the donor ($\geq 10^{10}$ mononuclear cells) as well as plasmapheresis (600ml plasma) and sent to MolMed as Fresh cells.

The donor cells will then be transduced by using the supernatant with retroviral vectors.

The transduced cells which express LNGFR will be selected with a specific antibody and immunomagnetic beads.

As per Amendment F at the end of the culture process the cells will be washed and resuspended ($25-100 \times 10^6$ cells/ml) in saline solution with human albumin 7%. DMSO 10% is used as a stabilizer for the cryopreservation. The cells are transferred into cryobags and into cryovials for QC tests and retention samples and are frozen using the programmable computer refrigerator ICE CUBE. Engineered frozen lymphocytes will be tested for viability, presence of mycoplasma, sterility EP and sterility hospital standard and bacteria and fungi, endotoxin, immunophenotype, LNGFR expression, RCR, GCV sensitivity, IL-2 dependent growth and TCR-V β analysis.

Depending on the clinical situation of the patient, the clinician may elect to infuse the product before the results of all QC tests become available; in this case, the infusion will take place at least 7 days after sample freezing, when all tests except IL-2 and sterility EP tests are completed. The results of the remaining tests will be forwarded at the clinician as soon as they become available.

The cryobag is transferred in dry ice to the clinical centres.

Before infusion the engineered T cells are thawed putting the cryobag into a water bath at 37°C.

The entire content of the cryobag is immediately infused after thawing.

3 Documentation of product tolerability

3.1 Tolerability of proteins encoded by the genes Δ LNGFR and HSV-tk

3.1.1 In vitro studies

The proteins ectopically expressed by lymphocytes genetically modified with HSV-tk and Δ LNGFR do not seem to alter either the proliferative potential or functionality of the cells.

In particular, repeated laboratory tests using genetically modified lymphocytes showed that the expression of these genes does not modify the phenotype of the cells and they continue, in fact, to express the characteristic markers of lineage, activation and adhesion.

There is no known difference between transduced and non-transduced cells in terms of proliferation and survival as evaluated by thymidine incorporation and spontaneous expression of annexin V,

suggesting that the introduction of new genetic information is neither an immortalizing and/or transforming event nor a spontaneous toxic factor for the cells.

As with non-engineered lymphocytes, the engineered cells maintain dependence on IL-2 for growth and survival. Deprivation of IL-2 causes the growth of the culture to slow down, inducing programmed cell death in the lymphocyte population^{47-52,58-62}

Regarding the specific introduction of the genes Δ LNGFR and HSV-tk in donor lymphocytes, we further add that to eliminate any possible functional activity of the marker LNGFR, the form with its intracellular domain truncated is used, and it is thus unable to transduce any signals (Δ LNGFR). The absence of any functional activity of the surface marker Δ LNGFR has been shown both in vitro and in vivo. The addition of the ligand (NGF) to lymphocytes expressing Δ LNGFR did not alter in vitro either the pattern of cellular proliferation or the secretion of cytokines⁵².

The protein expressed by HSV-tk, which confers ganciclovir sensitivity to donor lymphocytes, in the absence of ganciclovir does not induce any alterations in proliferation, lytic activity and cytokine production by transduced cells⁶³

3.1.2 In vivo studies

In almost ten years of clinical experimentation and another fifteen years of research in animal models, retroviral vectors have not shown any problems related to toxicity, side effects and safety of handling^{64,65}.

In particular, the risk of the occurrence of somatic mutations, potentially dangerous and linked to the insertion of a retroviral vector in unforeseen positions of the target cells genome, has been shown to be more theoretical than real. No such problem has ever been reported either in animals or in patients.

The efficacy of the suicide system based on HSV-tk/ganciclovir has been tested in different animal models. The following have been used:

□ The murine T lymphoma YC8 that, when inoculated in balb/c mice, causes solid tumors (after subcutaneous inoculation) and metastasis (after intravenous inoculation).

Up to 104 YC8 transduced and, therefore, expressing HSV-tk cells were infused subcutaneously and intravenously respectively into 2 groups of 10 balb/c mice per group. Five days after infusion, 5 mice/group received a dose of ganciclovir at 100 mg/kg/day intraperitoneally for 5 days.

The administration of ganciclovir significantly reduced the solid tumors size, as well as both the number and size of metastasis.

□ Hagenbeek et al, Department of Hematology, Utrecht has developed a model for GvHD in the Brown Norway (BN) rat. In this model, splenic T cells from Wag/Rij rats (donor) are transduced with the vector SFCMM-3 and then infused along with hematopoietic stem cells into the recipient BN rats after a conditioning regimen with TBI + ATG + FK506. This results in the development of acute GvHD; the administration of ganciclovir at the time of GvHD occurrence delays death due to GvHD from day 18-20 to day 28-30; furthermore, prophylactic administration of ganciclovir significantly augments the survival⁶⁶.

□ Apperley et al, Department of Hematology, Hammersmith H., London has developed a mouse model using RMA cells transduced with SFCMM-3. RMA cells transduced with SFCMM-3 are inoculated sc at doses of 105-106 in C57BL/6 mice. A cohort of mice is then administered ganciclovir at 20 mg/kg ip as prophylaxis the day of the cells inoculation while a second cohort is treated once tumor nodules reach 1 cm in diameter. The administration of ganciclovir prevents (in the first cohort) or reduces (in the second cohort) the tumor development⁶⁷.

□ Tiberghien et al, University Hospital Center (CHU), Besançon, has developed a model using balb/c and C57b1/6 mice transplanted with FBV stem cells with the addition of splenocytes from HSV-tk+ mice (derived from FBV mice transgenic for HSV-tk). At the occurrence of GvHD, the administration of ganciclovir augments survival of 40-60% in comparison to 0-6% of control mice⁶⁸.

□ Helene et al. has developed a mouse model for the administration of B10.A stem cells + 5x10⁶ B10.A (5R) splenocytes which are transgenic for HSV-tk to DBA/2 mice that have been lethally irradiated. In this model, the prophylactic administration of ganciclovir at days 3 and 10 reduces in the recipient mice the GvHD lethality from 50% to 0%⁶⁹.

4 Documentation of product efficacy

4.1 In vitro studies

Transduction Process

The sponsors of this study have ten years experience in genetic transfer via retroviral vectors in activated human lymphocytes.

Retroviral vectors integrate into cells in active replication, meaning that the human lymphocytes are activated in vitro with polyclonal mitogens and then infected with retroviral supernatant. The final product has been extensively studied in terms of immunophenotype, cytokine secretion and functional repertory. Lymphocytes that are transduced and cultivated show an inversion in the CD4/CD8 ratio, favoring the cytotoxic population, which is considered to be the effector cells in antitumoral and antiviral responses.

They show a cytokines spectrum favoring type 1 cytotoxic and helper lymphocytes (Tc1 e Th1), and, in the presence of IL-2, proliferative and lytic activities against allogeneic, viral and tumor antigens.

Lymphocytes genetically modified with SFCMM-3 maintain proliferative and cytotoxic activities equal to that of non-manipulated donor lymphocytes in vitro when tested against the following targets (relevant in the context of allogeneic transplantation):

allogeneic lymphocytes

autologous cell lines immortalized with Epstein Barr Virus (EBV-LCL)

autologous cell lines pulsed with peptide M58-64 from influenza virus

autologous fibroblasts infected with Cytomegalovirus.

Furthermore, stimulating non-manipulated cells or lymphocytes transduced and selected using autologous fibroblasts infected with Cytomegalovirus, the same antigen specificities are obtained, which indicates that lymphocytes against immunodominant Cytomegalovirus antigens are maintained in functional condition during the process o

ΔLNGFR

The presence of the surface marker ΔLNGFR permits rapid in vitro selection of transduced cells, via the use of magnetic beads conjugated with specific anti-LNGFR antibodies.

This immunoselection allows for the generation of a cell population almost totally transduced (purity >90%), that can be modulated in vivo with the administration of ganciclovir. The immunoselection, moreover, requires short cell culture times (an important parameter in order to best preserve the immune repertoire) and make it possible to obtain the number of cells needed for the clinical application. Furthermore, ΔLNGFR allows for rapid ex-vivo analysis and characterization of the cells, using double fluorescence studies.

HSV-tk

The HSV-tk protein confers donor T lymphocytes sensitivity to ganciclovir, in a reproducible dose-dependent manner resulting in a high mortality percentage⁴⁷.

Lymphocytes genetically modified with SFCMM-3, cultivated in the presence of ganciclovir for 5 days and exposed to tritiated thymidine and counted in a Beta counter, show a significant growth inhibition. This test reproducibly shows an inhibition in the proliferation of genetically modified cells greater than 90%. Similar results are obtained in terms of cell death using trypan blue to perform live cell counts. Therefore, the expression of HSV-tk permits a rapid and efficient elimination of genetically modified cells once infused into the patient. It should be taken into consideration that the suicide mechanism based on HSV-tk/ganciclovir represents a system used in other protocols of gene therapy to increase the safety of use of genetically modified cells and to allow their clearance in the event of adverse or undesirable reactions.

Recently, an internal splicing site of the HSV-tk gene has been described. This site is responsible for the generation of an mRNA containing a deletion of 227 bp, thus coding for a variant of HSV-tk that is not sensitive to ganciclovir⁷⁰.

In different clinical protocols, this variant has never been detected in more than 10% of the cells infused into the patient. However in the clinical grade supernatant that will be employed in this trial the spliced variant was below 2%. Even though 90% of the transduced cells are completely sensitive to ganciclovir, the group of investigators participating in this trial is developing a gene variant without the deletion.

4.2 Recent clinical experiences with lymphocytes genetically modified with HSV-tk

The rules that regulate the GMP production of vectors and packaging lines are, at present, well described. All the potential risks associated with the use of the technology and the quality control methods that allow for monitoring and control are well known⁷¹.

The packaging cell line that will be used in this study (GP+env AM12) is derived from the 3T3 already used worldwide. This packaging line⁵⁷ was the first to be approved by the NIH and FDA for clinical use. The development of RCR represents the principal potential risk associated with the clinical use of retroviral vectors. Data obtained in primates has demonstrated that even very high levels of RCR do not cause toxicity or pathogenicity⁷² and tumors occurrence is only caused in animals that are very immunodepressed⁷³.

The technologies available today for the RCR monitoring in both the packaging cell line and the ex vivo engineered tissues allow for a very effective quality control for the RCR presence, thus reducing the potential risks practically to zero even in immunodepressed patients. The clinical trials performed using engineered lymphocytes via retroviral vectors have never found any toxic reactions or side effects due to the retroviral vector, the engineering procedure or the exogenous protein^{44-48,58-60,74,75}.

This genetic manipulation does not, in any way, involve germinal cells and cannot be passed onto offspring.

The results published in the literature, regarding the infusion of lymphocytes genetically modified with HSV-tk, are encouraging in terms of absence of toxicity related to gene transfer, persistence in the circulation of transduced cells, antitumoral activity and especially in terms of effectiveness of the suicide strategy. In fact, it has always been possible – in case of GvHD occurrence – to completely or partially eliminate the cells transduced with HSV-tk via administration of ganciclovir, reducing or eliminating the GvHD symptoms^{45-47,60,74}.

The feasibility of this therapeutic approach in the context of allogeneic stem cells transplantation from a HLA compatible donor has already been demonstrated.

Data in the literature show, for example, that the infusion of lymphocytes transduced with HSV-tk in 8 patients, who underwent allogeneic transplantation and had subsequent disease relapse or EBV-induced lymphoproliferative disorders, did not result in any toxicity phenomena related to the in

in vitro manipulation of lymphocytes. The therapeutic activity (GvL) of genetically modified lymphocytes was observed in 5 out of 8 patients with 3 patients obtaining complete remission. The study seems to also suggest the possibility of controlling GvHD via the administration of ganciclovir. In fact, in 2 patients affected with acute GvHD post-infusion and treated with ganciclovir, both clearance of circulating lymphocytes and complete regression of GvHD were observed.

In one patient, who developed chronic GvHD, the infusion of ganciclovir allowed for a reduction of transduced lymphocytes and a partial clinical response, that resulted in a complete response after the co-administration of low doses of immunosuppressive drugs. Even though the number of patients is too low for statistical analysis, the results obtained in this study are very similar to those reported in the literature on the use of genetically modified lymphocytes, suggesting that the in vitro manipulation of these lymphocytes does not significantly alter their immunological activity^{45-49,58,59,74}.

Tiberghien at the University Hospital (CHU), Besançon treated 12 patients with escalating doses of cells transduced with HSV-tk and neo, as prophylaxis against disease relapse after HLA identical T-depleted marrow transplantation.

In this study, the presence of circulating genetically modified cells was shown in 10 out of 11 patients. Furthermore, it was possible to document that the level of genetically modified cells in circulation increased in correspondence with the GvHD occurrence. Six patients were treated with ganciclovir, 5 for GvHD, 1 for Cytomegalovirus infection; in 5 out of 6 patients ganciclovir treatment resulted in a significant reduction of the genetically modified cells number (92% reduction); the reduction of HSV-tk+ cells is comparable in patients that have just been infused or treated months previously, thus denoting the stability of vector expression. Of note is that both in this proposed trial and the Tiberghien study, the transcription of the HSV-tk gene is controlled by the same promoter (LTR of MoMuLV); consequently it is reasonable to hypothesize an identical expression pattern.

The treatment with ganciclovir obtained a complete remission of the GvHD signs in 2/3 patients with acute GvHD of a grade \geq II and in 1/1 patient with chronic GvHD. In one of the patients with acute GvHD, it was necessary to add steroid treatment for the complete control of GvHD signs. The reduction in the symptoms and signs of GvHD began 24 hours after the first administration of ganciclovir and was complete after about 1 week⁶⁰.

In a study conducted by Link and colleagues, in which lymphocytes genetically modified with HSV-tk and neo were administered for the treatment of disease relapse after transplantation, no adverse reactions attributable to lymphocytes were noted. In treated patients, the percent of circulating transduced lymphocytes ranged from 0 to 3.8 %. 3 patients responded to the infusion in terms of antitumoral activity and 3 did not respond (CML). One patient developed chronic GvHD that did not respond to conventional therapy but did respond to ganciclovir⁵⁹.

Champlin and colleagues conducted a Phase I/II study with lymphocytes genetically modified with HSV-tk for post-transplantation relapse. Twenty three patients were treated with 1-4 doses per patient (from 0.7 to 190×10^6 cells/kg).

In almost all the patients, it was possible to document the presence of circulating transduced cells, and there was no adverse reaction to the treatment. Two patients, affected by chronic myelogenous leukemia, had complete remission with a dose of 50 or 85×10^6 cells/kg, 4 remained stable and 17 had disease progression. Only one patient developed grade I GvHD for which no therapy was needed⁵⁸.

5 Objectives of the study

5.1 Primary objectives

Primary objectives of the trial are:

- ❑ Evaluation of clinical activity in terms of immune reconstitution after SCT
- ❑ Evaluation of the in vivo control of GvHD after administration of ganciclovir in patients treated with HSV-tk transduced cells
- ❑ Evaluation of GvL effect

The primary activity parameter for the evaluation of **immune reconstitution** are:

- Number of circulating CD3+ $\geq 100/\mu\text{l}$
- Number of circulating CD3+CD4+ $\geq 50/\mu\text{l}$
- Number of circulating CD3+CD8+ $\geq 50/\mu\text{l}$

The primary activity parameters for the evaluation of the **incidence/control of GvHD and GvL effect** are:

- ❑ Occurrence of GvHD and response to ganciclovir
- ❑ Number of circulating transduced cells after treatment with ganciclovir
- ❑ Evaluation of GvL effect by clinical, radiological, molecular, hematological and cytogenetical criteria

5.2 Secondary Objectives

Secondary objectives of the trial are:

- ❑ Disease free survival and overall survival
- ❑ Incidence of infectious events
- ❑ Acute and long-term toxicity related to the infusions

The parameters for calculation of the secondary end points are:

- ❑ Time to relapse, time to death (disease free survival and overall survival)
- ❑ Number of infectious events
- ❑ Incidence of adverse events

6 Study design

6.1 Study plan

This is a phase I-II open study, non randomised, multicentric and international, to be conducted in 4-5 Institutions.

The following steps are foreseen:

- Leukapheresis of the donor, transduction and selection of lymphocytes using the retroviral vector SFCMM-3
- Monitoring of the patient after SCT to evaluate the level of lymphocytes subsets and immune reconstitution. Monitoring of the immune reconstitution will be evaluated for the whole trial according to the schedule of assessment

□ First Infusion

Administration of genetically modified donor lymphocytes at a dose of 1×10^6 or 1×10^7 cells/kg, is planned in the absence of spontaneous immune reconstitution (documented by two consecutive findings of circulating CD3+ cells $\geq 100/\mu\text{l}$) and/or development of GvHD.

In general, the infusion(s) must be performed within the interval between day+21 and day +49 after SCT (except for patients under therapy with ganciclovir due to cytomegalovirus; in these cases the lymphocytes infusion must be performed 24 hours after the discontinuation of ganciclovir).

Delays in the infusion(s) administration due to technical reasons must be documented and reported in the case report form (CRF).

In the event of patients transplanted in relapse or in relapse after transplant, clinical centers can apply for permission to either start treatment with a dose different from the first dose scheduled in the protocol. In any case this should be approved by the sponsor through submission of appropriate documentation on disease status.

□ Second Infusion

30 days after the first infusion, in the absence of both active GvHD and immune reconstitution, documented by two consecutive findings of circulating CD3+ cells $\geq 100/\mu\text{l}$, genetically modified lymphocytes will be infused at a dose of 1×10^7 cells/kg.

□ Third Infusion

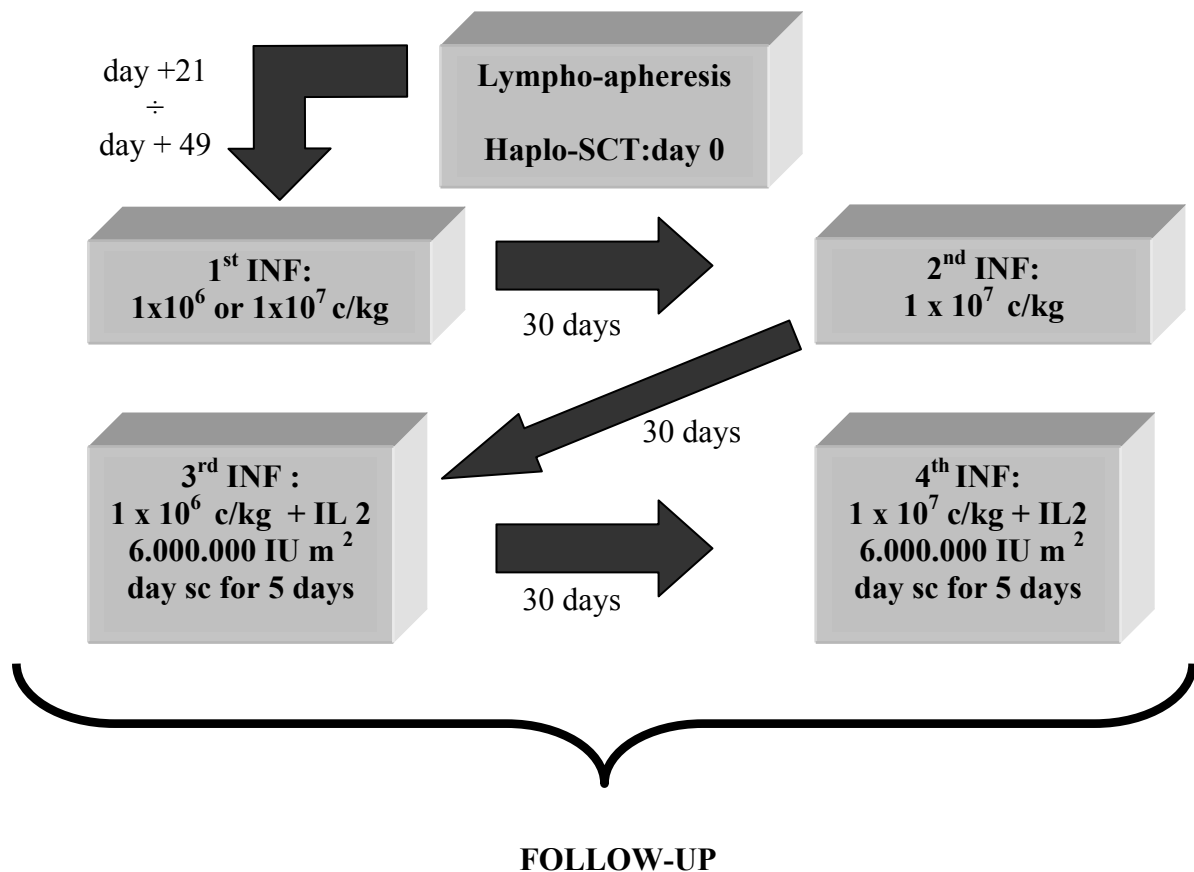
30 days after the second infusion in the absence of both active GvHD and immune reconstitution, documented by two consecutive findings of circulating CD3+ cells $\geq 100/\mu\text{l}$, genetically modified lymphocytes will be infused at a dose of 1×10^6 cells/kg in combination with interleukin 2 at dosage of 6.000.000 IU/m² sc per 5 days.

□ Fourth Infusion

30 days after the third infusion in the absence of both active GvHD and immune reconstitution, documented by two consecutive findings of circulating CD3+ cells $\geq 100/\mu\text{l}$, genetically modified lymphocytes will be infused at a dose of 1×10^7 cells/kg in combination with interleukin 2 at dosage of 6.000.000 IU/m² sc per 5 days.

□ Donor Lymphocytes Infusions (DLI)

In the event of patients transplanted in relapse the follow up phase (6 month) or after the 6th month, the clinical center can apply for permission to a DLI. In any case, this should be approved by the Sponsor through submission of appropriate documentation on disease status and appropriate rationale for the performance of DLI.



7 Study population

7.1 Inclusion criteria

- Patients > 18 years old affected by hematological malignancies at high risk of relapse based on disease progression or presence of negative prognostic factors, who have received a SCT from a HLA mismatched (haploidentical) donor for 2 or 3 loci
- Engraftment verified by >500 neutrophils/ μ l for three consecutive days in the absence of growth factors
- Mixed chimerism or full donor chimerism confirmed
- AML in 1st or 2nd relapse or primary refractory
- High-risk AML in 1st or subsequent remission
- RAEB and RAEB-T
- CML in 2nd chronic phase, blast crisis or accelerated phase
- Poor prognosis ALL in 1st or subsequent remission
- High grade lymphomas in 3rd or subsequent remission
- Multiple myeloma in advanced stage relapsing or progressing after high dose chemotherapy
- Absence of fully HLA matched or one HLA locus mismatched family donor
- Stable clinical conditions and life expectancy > 3 months
- PS Karnofsky >70
- Written donor/patient informed consent

7.2 Exclusion criteria

- Infection with Cytomegalovirus being treated with ganciclovir
- Presence of GvHD grade > I that requires systemic immunosuppressive therapy
- Ongoing systemic immunosuppressive therapy
- Ongoing acyclovir administration
- Administration after SCT of G-CSF and cyclosporine A
- CD3+ lymphocytes >100/ μ l before infusion
- Patients with life-threatening condition or complication other than their basic disease.
- Patients with CNS disease
- Pregnant or lactating women

7.3 Conditioning regimen

The recommended conditioning regimen foresees the following steps:

7.3.1 Total body irradiation (TBI) containing regimen

- | | |
|---|---------------|
| ▪ TBI 7.5 Gy, single fraction | day -9 |
| ▪ Thiotepa 10 mg/kg/die iv splitted in two 4 hours infusions (5 mg/Kg/q12h) | day -8 |
| ▪ Fludarabine 40 mg/sqm/die iv | days -7 to -3 |
| ▪ ATG- Merieux 3 mg/kg/die or Fresenius 5 mg/Kg/die | days -6 to -2 |
| ▪ Rest | day -1 |

7.3.2 Conditioning regimen without TBI

- Melphalan 140 mg/sqm day -9
- Thiotepa 13 mg/kg/die splitted in two 4 hours infusions day -8
(6,5 mg/Kg/q12h)
- Fludarabine 40 mg/sqm/die iv days -7 to -3
- ATG- Merieux 3 mg/kg/die or Fresenius 5 mg/Kg/die days -6 to -2
- Rest day -1

7.3.3 Exclusion criteria for TBI

- Age >40.
- Previous TBI or involved field (loco-regional) irradiation.
- History of systemic mycosis.
- History of lung lesions without proved bacterial origin.
- Positive aspergillus screening test.
- Prolonged continuous treatment with steroids in the previous year.
- Neutropenia <100 N/ μ L in the previous 4 weeks

7.3.4 Reduced toxicity conditioning regimen

Reduced toxicity conditioning regimen aimed at improving the toxicity profile could be indicated for a subset of patients affected by lymphoid or myeloid haematological disease, with age >55 and/or presenting with associated comorbidity or organ impairment.

The following regimen is indicated for lymphoid neoplastic haematological disease.

- Thiotepa 10 mg/kg/die iv splitted in two 4 hours infusions day -9
(5 mg/Kg/q12h)
- Rituximab 375 mg/sqm iv (8 hours) days -8 and -2
- Fludarabine 30 mg/sqm/die iv (1 hour) days -7 to -3
- ATG-Fresenius 5 mg/kg/die iv (16 hours) days -7 to -3
- TBI 200 cGy, single fraction day 0

The following regimen is indicated for myeloid neoplastic haematological disease

- Treosulfan 14 mg/sqm iv days -6 to -4
- Fludarabine 30 mg/sqm/die iv (1 hour) days -6 to -2
- ATG-Fresenius 5 mg/kg/die iv (16 hours) days -6 to -2
- Rituximab 375 mg/sqm iv (8 hours) day -1
- TBI 200 cGy, single fraction day 0

8 Procedures and treatment plan

8.1 Screening and baseline phase

The patient who enrolled in the study will be evaluated in the following way:

The screening phase will be performed before the transplant of stem cells (pre-SCT) and in a timeframe of 30 days (-30 to -1). The collection of data during the screening phase will allow for the control of the conditioning regimen as well as the evaluation of safety related to the administered agents. The screening phase will include:

- ❑ Informed Consent
- ❑ Medical history and complete objective examination
- ❑ Laboratory examinations:
 - haematology
 - liver function
 - renal function
 - electrolytes dosage
 - glycemia
 - total protein
 - immunoglobulin dosage
- ❑ CMV viremia and/or antigenemia
- ❑ HCV serology
- ❑ HBV serology
- ❑ EBV serology
- ❑ Bone marrow aspiration for evaluation of the neoplastic disease
- ❑ Cytogenetics and molecular tests of bone marrow and peripheral blood for markers of disease
- ❑ Chest X-ray and possible further diagnostic tests aimed at evaluating the disease
- ❑ Spirometry with DLCO

The baseline phase will be performed after the transplant (post SCT) and in the 10 days before the first planned infusion at day +42 (from day +30 to day +40).

Collection of data during the screening/baseline phase will allow for the evaluation of peri transplant mortality and safety. It will include:

- ❑ Complete objective examination with particular attention for clinical signs of GvHD
- ❑ Possible liver, cutaneous or mucosal biopsies in case of clinical suspicions of GvHD
- ❑ Laboratory examinations: hematology, immunonophenotype, complete liver function; electrolytes dosage; glycemia; total protein and immunoglobulin dosage.
- ❑ CMV serology; viremia and/or antigenemia for CMV
- ❑ Bone marrow aspiration for evaluation of the disease progression
- ❑ Cytogenetics and molecular tests of bone marrow and peripheral blood for markers of disease and evaluation of the degree of chimerism between donor/host
- ❑ FACS analysis for the expression of LNGFR by the neoplasm.
- ❑ PCR for tk
- ❑ Confirmation of mixed or full chimerism
- ❑ Chest X-ray, possible further diagnostic tests aimed at the disease evaluation

| BASELINE PHASE | | |
|-----------------------------|---|--|
| | Within 10 days before 1 st INF | |
| Stem Cell Transplant | <p>Complete objective examination with particular attention for clinical signs of GvHD.</p> <p>Possible liver, cutaneous or mucosal biopsies in case of clinical suspicions of GvHD.</p> <p>Laboratory examinations: hematology, immunonophenotype, complete liver and renal function; electrolytes dosage; glycemia; total protein and immunoglobulin dosage.</p> <p>CMV serology; viremia and/or antigenemia for CMV;</p> <p>Bone marrow aspiration for evaluation of the disease progression.</p> <p>Cytogenetics and molecular tests of bone marrow and peripheral blood for markers of disease and evaluation of the degree of chimerism between donor/host.</p> <p>FACS analysis for the expression of ΔLNGFR by the neoplasm. PCR-tk</p> <p>Chest X-Ray, possible further diagnostic tests aiming at the disease evaluation.</p> | 1st Infusion of HSV-tk T-cells |

8.2 Evaluation of the donor and leukapheresis

The donors will be selected according to the national legislation.

The peripheral blood stem cell harvest will be done prior to initiating the conditioning regimen. CD34 positive selection should be performed with the Clinimacs device. Donor stem cells will be mobilised with G-CSF. A recommended minimum of 7×10^6 CD34+/kg stem cells will be cryopreserved following local protocols. The graft composition should be adjusted to contain a dose close to 1×10^4 /kg CD3+ lymphocytes.

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 leukapheresis, upon donor informed consent. The leukapheresis will then be delivered to MolMed S.p.A. or to other GMP facilities included in the trial, and manipulated as previously specified in chapter 2.3. In the event that a further lymphocytoapheresis is needed, it will be done at least 30 days after administration of G-CSF. Every effort should be made when choosing the haploidentical family donor to select a donor/recipient combination with a donor anti-recipient KIR incompatibility.

8.3 Infusion of donor lymphocytes engineered with HSV-tk

Engineered donor T-lymphocytes with SFCMM-3 retroviral vector will be infused intravenously into the patient previously medicated with clorpheniramine at dosage of 10 mg/iv. The product will be provided to the investigator in ethylene-vinyl-acetate infusional bags.

8.4 Treatment of GvHD

The classification of GvHD is described in Appendix A.

If at any time during the study, a GvHD equal to or greater than II develops, the patient will be treated with ganciclovir at dosage of 10 mg/kg/day divided into two administrations for 14 days.

In case of GvHD progression after three days of treatment only with ganciclovir, a standard immunosuppressive therapy will be added. An early stopping rule applicable to the onset of GvHD is described in chapter 12.6.

9 FOLLOW-UP

9.1 Immunological Studies

Part of the immunological studies aimed at verifying the status of the immune reconstitution will be conducted locally at each site while part of the experiments aimed at characterizing the immune reactivity of transduced cells will be conducted as centralized procedures at HSR.

PCR-tk and RCR will be performed centrally at MolMed S.p.A. laboratories.

9.1.1 Schedule of immunological studies

The patients will be evaluated for immunological follow-up starting after the first (or subsequent) infusion according to the described schedule. In the event of additional infusions after the first, as planned in the protocol, the frequency of the examination will restart from the beginning (1st month). For a better comprehension the dates of immunological studies have been reported as days from each infusion (day 14th, 21st, 28th at 1st month, at day 14th for the second, third, fourth, fifth and 28th at sixth month of follow up); these time-points also correspond (in presence of a regular schedule) to day 44th, 74th, 104th, 134th and 180th after each infusion.

| Schedule of Immunological Studies | | | | | | |
|-----------------------------------|-----------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Month (from infusion) | 1st | 2nd | 3rd | 4th | 5th | 6th |
| Frequency | Day 14,21,28 | Monthly (day 14) | Monthly (day 14) | Monthly (day 14) | Monthly (day 14) | Monthly (day 28) |

a) The following studies will be performed **locally** at each site:

- Flow cytometry: **CD3-PE, CD3-PE/CD4-FITC, CD3-PE/CD8-FITC** (ISHAGE protocol) on CD45+ cells (CD45-PerCP), to evaluate the number of circulating cells/ μ l
- Flow cytometry: % of lymphocytes on the total leukocytes, as assessed by physical parameters and n° of circulating lymphocytes/ μ l.
- Flow cytometry: **IgG1-FITC/IgG1-PE, CD2-PE/CD19-FITC, CD16-FITC/CD56-PE, CD3-FITC/HLA-DR-PE**, expressed as % of cells gated on physical parameters of lymphocytes and on CD45+ cells (CD45-PerCP).
- Flow cytometry LNGFR:
 - on a healthy donor's sample: **RAM-FITC, LNGFR+ RAM-FITC.**
 - on patient's sample: **RAM-FITC, LNGFR+RAM-FITC.**

Data will be expressed as % of cells gated on physical parameters of lymphocytes. % of Δ LNGFR+ cells will be expressed as the difference of the % of Δ LNGFR+ cells measured on the patient's sample minus the % of Δ LNGFR+ cells measured on the healthy donor's sample. Total n° of circulating Δ LNGFR+ cells/ μ l will be calculated.

□ **When LNGFR+ cells are equal or exceed 2,5%** of circulating lymphocytes, the following cytofluorimetric analysis will be performed locally:

- **RAM-FITC/IgG1-PE**
- **LNGFR+RAM-FITC/CD3-PE**
- **LNGFR+RAM-FITC /CD4-PE**
- **LNGFR+RAM-FITC /CD8-PE**
- **LNGFR+RAM-FITC /CD16-PE**

Data will be expressed as % of cells gated on physical parameters of lymphocytes. Total number of circulating Δ LNGFR+ subpopulations/ μ l will be calculated.

This analysis will be started only when LNGFR+ **cells are equal or exceed 2,5%** of circulating lymphocytes and will be performed from then on with the same schedule used for the other phenotypic analysis.

All flow cytometry events (and not only gated events) will be saved in a file and made available to the sponsor for a peer review at the end of the study. A minimum of 100 positive events will be acquired for every sample.

b) The following evaluation will be **centrally** (HSR and MolMed) performed on PBMC isolated from the indicated samples:

□ **When LNGFR+ cells exceed 2,5%** a further 50 ml of blood will be requested. These samples (label CH/2,5%) **are requested only ONCE during the study** and will be sent to MolMed. The following studies will be performed at **HSR**

- Cytofluorimetric analysis for the surface markers, CD16, HLA-DR, CD25, CD45RA, CD45RO, CD27, CCR7 to evaluate the relative in vivo expansion of different T cell subpopulations
- Analysis of the transduced T cell subsets surviving in vivo (TH1 TH2, TC1, TC2 T regulatory cells) by intracytoplasmatic staining
- Detection of antigen-specific transduced T cells by the use of tetramers specific for CMV peptide restricted in HLA-A2, EBV peptide restricted in HLA-A2 (available for HLA-A2+ donors)

□ **When LNGFR+ cells exceed 5%** of circulating lymphocytes, a further aliquot of 50 ml of blood will be requested; these samples (label CELL SORTING) are **requested only ONCE during the study** will be sent to MolMed and the following studies will be performed at HSR:

- cell sorting for PCR analysis(TCR V β subfamilies)
- cell sorting for sensitivity to ganciclovir

□ **At baseline, when circulating CD3+ cells exceed the level of 100/ μ l and at the end of study**, additional 50 ml of blood will be sent to MolMed. The following studies (samples with the correspondent label functional studies) will be performed at HSR when CD3+ cells exceed the value of 100/ μ l:

- Molecular analysis for rearrangement of the T cell receptor
- Response to polyclonal mitogens
- Analysis of the cytokine pattern produced by the lymphocytes
- Proliferation in the presence of antigens such as CMV, EBV
- Cytotoxic activity when exposed to cells expressing antigens such as CMV, EBV

□ **In case of unexpected disappearance** of transduced donor lymphocytes from circulation, it will be investigated if an immune response to the transgene has occurred

9.2 Real time pcr-tk (polimerase chain reaction)

PCR analysis for HSV-tk aims at verifying the presence of transduced donor lymphocytes (% of TK wt cells vs spliced positive cells). The analysis will be performed at the following timepoints:

- baseline
- at day 14-21-28 during the first month of follow up
- at day 14 during the second, third, fourth and fifth month of follow up
- at day 28 during the sixth month of follow up

It is needed 10 ml blood (label PCR-TK). In order to correctly perform the examination at least 5×10^6 PBMC post Ficoll are requested. Double volumes are requested at baseline and during the 1st month of follow up after each infusion. The samples will be sent to MolMed.

9.3 Retrovirus competent for replication (RCR)

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

- pre-post infusion,
- at 3 months,
- at 6 months,
- at one year
- yearly, as suggested by the FDA guidelines (Guidelines for Industry, November 1999).

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

It is generally needed 10 ml blood (label RCR) in order to correctly perform the examination at least 5×10^6 PBMC post Ficoll are requested. During each pre-post infusion time point, double volumes are requested.

9.4 Immunological studies in presence of GvHD

In presence of GvHD a 50 ml plus a 10 ml of blood in the pre ganciclovir phase, after 4 days of administration and at the day after the discontinuation of ganciclovir will also be requested for each time point (correspondent label PRE GCV, AFTER 4 DAYS GCV, POST GCV). The samples will be sent to MolMed.

9.5 Immunological studies in presence of ganciclovir administration

In case of Ganciclovir administration (administered for CMV reactivation) a 10 ml of blood will be requested at the following time points: before treatment with ganciclovir; 4 days after beginning treatment and the day after interrupting ganciclovir (label PRE GCV, AFTER 4 DAYS GCV, POST GCV). The samples will be sent to MolMed.

9.6 Follow up in case of premature discontinuation from the study

In case of discontinuation of treatment due to spontaneous refusal, severe toxicity or progression of disease the patient must be followed for overall survival analysis. All potential delayed adverse events or lab values modifications or serious adverse events during this timeframe will be immediately notified to MolMed S.p.A as the patient was formally active in the study and according to chapter 11.1 using the SAE form enclosed with the CRF.

9.7 Follow up in case of Donor lymphocyte infusion (DLI) for treatment of disease relapse

In case of administration of Donor Lymphocyte Infusion(s) to treat disease relapse, the patient must be followed for overall survival analysis. All potential delayed adverse events or lab values modifications or serious adverse events during this timeframe will be immediately notified to MolMed S.p.A as defined in the chapter 11.1 using the SAE form enclosed with the CRF.

The following evaluations will be requested according to the schedule:

PCR-tk: pre DLI infusion and monthly for 6 months

Immunophenotype: monthly for 6 months

RCR: pre-post infusion, at 3,6,12 months and yearly

9.8 Non immunological follow up

The patients will also be evaluated for non-immunological follow-up (including physical examination, standard hematology, chemistry and imaging) after the first infusion (or subsequent infusion).

The overall evaluation (in accordance to Chapter 22 FLOW CHART) will include:

- complete objective examination with particular attention for clinical signs of GvHD. In case of GvHD biopsies must be obtained and evaluated for proving GvHD, and other potential pathologies, analyses will use immunohistochemical studies for the cell surface marker Δ LNDR and molecular analysis using PCR for HSV-tk, in order to assess the presence of genetically modified cells at GvHD sites.
- laboratory examinations: hematology, hepatic and renal functions, electrolytes dosage, glycemia, total proteins, immunoglobulin dosage, viremia and/or antigenemia for CMV.
- disease relapse/progression will be evaluated via morphological examination of the marrow, cytogenetic analysis and molecular exams of the marrow and peripheral blood together with suitable instrumental imaging (when appropriate) diagnostic examinations.
- adverse events collection (throughout the trial).
- analysis and characterization of the infused cells.

9.9 Biologic material to be collected during the study

Samples of bone marrow (4 ml) and peripheral blood (**buffy coat obtained from 350-400 ml of peripheral blood**) from the patient **before Stem Cell Transplant** as well as the samples of peripheral blood collected during the study and necessary for the centralized analysis will be processed and frozen in every center and made available to **MolMed/HSR**. Processing will consist in PBMC isolation (pool all the blood sample at each time point) by density gradient centrifugation. Appropriate labels will be provided in order to identify each samples.

9.9.1 Blood collection

Each sample will be collected into a glass violet top vacutainer tube bearing an appropriate label.

10 ml blood sample should allow to collect at least 5×10^6 PBMC/cryotube whereas the 50 ml blood sample should allow approximately 20×10^6 PBMC/vials split into 2 vials.

Each sample of collected PBMC will be harvested and frozen as viable cells. MolMed will organize the shipping of stored samples.

9.9.2 Summary of blood sample volumes required

| | | |
|---|--|--------------|
| PERIPHERAL BLOOD PRE-SCT | | 50 ml |
| PRE-SCT BONE MARROW | | 4 ml |
| | | 10 ml |
| PCR-tk | (20 ml at baseline and during the first month) | 10 ml |
| RCR testing | (20 ml at each pre post infusion) | 10 ml |
| CH/2.5 | | 50 ml |
| CELL SORTING | | 50 ml |
| PRE-GANCICLOVIR | | 10 or 60* ml |
| GANCICLOVIR AFTER 4 DAYS TREATMENT | | 10 or 60* ml |
| POST-GANCICLOVIR | | 10 or 60* ml |
| FUNCTIONAL STUDIES | | 50 ml |

* in case of GvHD

10 TREATMENT

10.1 Experimental product

Density gradient isolation and subsequent freezing of the mononuclear cells will be performed by MolMed S.p.A or by GMP facilities present at the clinical sites participating in the trial.

Before each planned infusion a proportional amount of cells will be thawed and transduced.

Cryopreserved engineered T-cells in NaCl physiological solution, albumin 7% and DMSO 10% at concentration of $25-100 \times 10^6$ /ml will be provided in ethylene-vinyl-acetate bags for infusion.

Before infusion the engineered T cells will be thawed putting the cryobag into a water bath at 37°C. The entire content of the cryobag will be infused immediately after thawing.

10.2 Outcomes after infusion of hsv-tk transduced lymphocytes

Following the infusion of each dose of HSV-tk transduced lymphocytes the following outcomes are expected:

1. The patient experiences immune-reconstitution (defined as a number of circulating CD3+ cells $\geq 100/\mu\text{l}$ for two consecutive observations) regardless of the presence or absence of any degree of GvHD. In this case, the patient receives no further infusion of HSV-tk transduced lymphocytes and is followed-up according to the study plan.
2. The patient fails to achieve immune-reconstitution and does not develop grade \geq II GvHD of any system within 30 days of the preceding infusion of HSV-tk transduced lymphocytes. In this case, the patient should receive the next planned dose of HSV-tk transduced lymphocytes according to the study plan.
3. The patient fails to achieve immune-reconstitution but experiences grade \geq II GvHD of any system not responding to Ganciclovir or other immunosuppressive therapy within 30 days of the preceding infusion of HSV-tk transduced lymphocytes. In this case, the patient receives no further infusion of HSV-tk transduced lymphocytes and is followed-up according to the study plan.
4. The patient fails to achieve immune-reconstitution but experiences grade \geq II GvHD of any system, which responds to Ganciclovir or other immunosuppressive therapy within 30 days of the preceding infusion of HSV-tk transduced lymphocytes. In this case, the safety committee (including all principal investigators) will make a decision as to whether to stop or to proceed with the previous or next planned dose of HSV-tk transduced lymphocytes according to the study plan. This decision will take place as soon as possible taking into consideration all the case circumstances.

10.3 Concomitant therapy associated with t-lymphocytes infusion

Increase in cell dose as well as administration of IL 2 (6.000.000 UI/sc/sqm per 5 days) will be allowed in the absence of immune reconstitution and in accordance with the study plan (chapter 6.1).

In case of GvHD ganciclovir will be administered at a dosage of 10 mg/kg/day.

10.4 Tolerability of interleukin 2

Interleukin 2 is a glycoprotein produced by T-helper lymphocytes under stimulation of mitogens or specific antigen. The biological role of IL-2 includes long term growth of T-lymphocytes cultures and mediation of important immunoregulatory in vivo and in vitro effects.

Experimental and clinical data showed the safety profile of the cytokine which is mostly related to the iv route with particular relevance to the cardiovascular system.

Hypotension was the most common effect, reported in about 65% of the patients treated and requesting pharmacological treatment and fluid administration.

Dyspnea was reported in 9% of the patients and in almost all the cases a specific treatment was required. Pleural effusion ascites and peripheral oedema were present in 4% of the patients treated; about one third of the patients showed weight increase > 10% in comparison to the baseline.

Angina or myocardial ischemia were reported in 3% of cases and myocardial infarction in 1% of the patients.

The toxicity threshold is represented by the onset of cardiovascular symptoms; these symptoms were similar to those observed during septic shock and are related to a capillary leak syndrome. Reduction in vascular peripheral resistance, followed by tachycardia and hypotension, reduction of plasmatic volume and oliguria, sodium retention, increase in serum creatinine, BUN are due to the transfer of albumin into the extravascular compartment.

This passage is probably due to functional modification of the endothelial barrier (direct action of IL-2 or cytokine mediated process). However, most of the mechanism underlying this syndrome are not completely clear.

In some cases neuropsychiatric symptoms were observed. Even if the symptomatology is known to be generally related to a pre-existing neoplastic lesion at the CNS, there was some evidence of interaction between IL-2, beta-endorphine, ACTH and cortisol.

IL-2 is able to pass hematoencephalic barrier. However, this passage might become excessive in case of partial damage of the barrier by a neoplastic lesion. The symptoms are similar to that observed during brain oedema, in certain cases of a severe degree such as coma and of difficult treatment.

Therapy with IL-2 is therefore contraindicated in presence of secondary neoplastic lesions.

Further adverse events of hematological type have been described (anemia, thrombocytopenia, lymphocytopenia) and the mechanism is most likely due to a redistribution of the lymphocytes with subsequent lymphocytosis at the end of the treatment itself.

During subcutaneous administration of IL-2 the incidence and severity of the adverse events are of a lesser degree when compared with the intravenous infusion.

In general only a mild degree systemic toxicity is reported, including fever, nausea and vomiting.

10.5 Tolerability of gancyclovir

Ganciclovir is commonly used in the field of congenital and acquired pathologies causing immunodepression, for the prevention and therapy of cytomegalovirus reactivation. The dose used for the treatment of GvHD in patients infused with lymphocytes genetically modified with HSV-tk, equal to 10 mg/kg/day, is the dose used in the treatment of Cytomegalovirus infections, with reduced dosage in the event of impaired renal function. The use of ganciclovir can be accompanied by side effects such as marrow depression, gastrointestinal toxicity and impaired renal function. However these side effects occur especially after long term administration, a different administration condition as compared with this study.

11 Safety and tolerability

An Adverse Event (AE) is any untoward medical occurrence in a patient or clinical investigation subject treated with a pharmaceutical product or a therapeutic agent and which does not necessarily have to report a casual relationship with this treatment. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding for example), symptom or disease temporally associated with the use of a medicinal product or therapeutic agent whether or not considered related to the product/agent. Pre-existing conditions which worsen during a study are to be reported as adverse events.

All the clinical adverse events encountered during the trial will be reported on the AE page of the CRF. The intensity of the clinical adverse event will be graded according to the National Cancer Institute Common Toxicity Criteria grading system (Appendix B).

Adverse reactions and their relationship with the cellular products infused during the study, concomitant treatments and blood derivatives possibly administered will be registered in the proper case report form for the entire duration of the study according to chapter 11.1. Laboratory test will be recorded on the laboratory results pages of the CRF.

A **Serious Adverse Event (SAE)** is any experience that suggests a significant hazard, contraindication side effect or precaution. With respect to clinical experience, this includes any experience which:

- is fatal
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/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.

For all serious adverse events, the following must be assessed and recorded on the adverse pages: intensity/severity, relationship to study drug, action taken regarding study drug (or investigational agent) and outcome to date.

A death occurring during the study or which comes to the attention of the investigator, whether considered treatment related or not, must be reported according to chapter 11.1. Such preliminary reports will be followed by detailed descriptions later which will include copies of hospital case reports, autopsy reports and other documents when requested and applicable.

The investigator must notify the Ethics Review Committee/Institutional Review Board of such an event in writing as soon as is practical and in accordance with international and local laws and regulations.

Any clinical adverse event or abnormal laboratory test value that is considered serious and occurring during the course of the study, irrespective of the treatment received by the patient must be reported to MolMed S.p.A according to chapter 11.1 and the local guidelines within **one working day** of occurrence to the following address and using the appropriate SAE form:

Antonio Lambiase MD, Director of Clinical Department MolMed S.p.A. via Olgettina 58, 20132 Milan, Italy

Phone 02/21277232 – Fax 02/21277239 e mail antonio.lambiase@molmed.com

Adverse events not listed on the NCI CTC grading system will be graded on a four point scale and reported in detail on the CRF as indicated:

- Mild: discomfort noticed but no disruption of normal daily activity
- Moderate: discomfort sufficient to reduce or affect daily activity
- Severe: inability to work or perform normal daily activity
- Life threatening: represents an immediate threat of life

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

Probable: this category applies to those adverse events which are considered with a high degree of certainty to be related to the test drug. An adverse event may be considered probable if:

- It follows a reasonable temporal sequence from administration of the drug
- It cannot be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors or other modes of therapy administered to the subject
- It disappears or decreases on cessation or reduction in dose
- It follows a known pattern of response to the suspected drug
- It reappears upon rechallenge

Possible: this category applies to those adverse event in which the connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An adverse event may be considered possible if or when:

- It follows a reasonable temporal sequence from administration of the drug
- It may have been produced by the subject's clinical state environmental or toxic factor's or other modes of therapy administered to the subject
- It follows a known pattern of response to the suspected drug

Unlikely: In general this category is applicable to an adverse event which meet the following criteria

- It does not follow a reasonable temporal sequence from administration of the drug
- It may readily have been produced by the subject's clinical state environmental or toxic factors or other modes of therapy administered to the subject
- It does not follow a known pattern of response to the suspected drug
- It does not reappear or worsen when the drug is readministered

Unrelated: This category is applicable to those adverse events which are judged to be clearly and incontrovertibly due only to extraneous causes (disease, environment...) and do not meet the criteria for drug relationship under remote, possible or probable

Definitely: This category is applicable to those adverse events which are judged to be clearly and incontrovertibly due only to the treatment (experimental or overall).

11.1 Safety flow chart

The collection of SAEs and AEs is phase related.

During the screening phase and until the administration of the 1st infusion, SAEs and AEs only related to infectious episodes will be collected to clearly define the safety during the peri transplant phase.

| | | |
|----------------------|--------------------------------|-------------------------|
| MolMed S.p.A. | CLINICAL STUDY PROTOCOL | Internal Code: IPR/01 G |
|----------------------|--------------------------------|-------------------------|

During the Active Treatment phase (which includes the treatment and the 6 months follow up phase) ALL SAEs and AEs will be collected regardless of their relationship with the experimental product.

After the follow up phase, a pharmaco-surveillance program addressed to register only product related SAE and product related AEs will be performed.

The protocol treatment schedule foresees also possible administration of DLIs in case of disease relapse at any time during or after the follow up phase.

Reporting of all potential SAEs and AEs are requested until the completion of the original 6 month follow up phase while only product related SAEs and AEs are requested during the follow up and beyond follow up phase.

This program will allow to detect and report potential delayed toxicity of the product and will be extended until exitus of the patient (included in the survival analysis) or until possible marketing of the product.

| Study Phase | SAE | AE |
|---|-------------------------------------|-------------------------------------|
| From screening until 1 st infusion | Only related to infectious episodes | Only related to infectious episodes |
| Active treatment phase (from 1 st infusion until 6 th month of follow up) | x | x |
| Post follow up | Only product related | Only product related |
| In case of DLI (during the 1 st month) | x | x |
| In case of DLI (during follow up or post follow up phase) | Only product related | Only product related |

11.2 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 30 days from the last administration of product/drug, she/he must immediately inform the investigator.

In case of pregnancy the 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 foetus. The patient will be monitored until the conclusion of the pregnancy.

11.3 Follow up in case of premature discontinuation from the study (Drop out)

In case of discontinuation of treatment due to spontaneous refusal, severe toxicity or progression of disease the patient must be followed to detect and report all potential delayed adverse events or lab values modifications according to chapter 11.1.

11.4 Follow up in case of donor lymphocytes infusion for disease relapse

The protocol treatment schedule foresees also possible administration of DLIs in case of disease relapse at any time during or after the follow up phase.

Reporting of all potential SAEs and AEs are requested for the first month after infusion while only product related SAEs and AEs are requested during the follow up and post follow up phase according to chapter 11.1.

12 Statistical considerations and analytical plan

12.1 Sample Size

The sample size was calculated using Simon's two-stage design method⁷⁶. The method provides the number of patients to enroll in the first (n_1) and second stage (n) of the study. To apply this method one must specify: a target rate of interest (P_1), a rate of no interest (P_0), the levels of α (type 1 error) and β (type 2 error). The decision of whether or not to terminate after the first stage will be based on the number of responses observed for those n_1 patients. The study will terminate at the end of the first stage if r_1 or fewer responses are observed. The probability that it will occur is denoted by PET_P_0 (probability of early termination). If the responses are greater than r_1 the study will continue until the n -th patient is enrolled. The therapy will be rejected at the end of the second stage if r_2 or fewer responses are observed. The probability that it will occur is denoted by PET_P_1 . If the number of responses at the end of the second stage is greater than r_2 the therapy under study will be considered a success.

Optimum design ($\alpha=0.05$, $\beta=0.10$)

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

The table above shows the calculation of n , n_1 , r_1 , r_2 after fixing P_0 (rate of immune-reconstitution under the hypothesis that the therapy is not efficacious) equal to 0.15, P_1 to 0.5 (rate of immune-reconstitution under the hypothesis that the therapy is efficacious) and α and β to 0.05 and 0.10, respectively.

Simon's method suggests that 7 patients have to be enrolled in the first phase. If the number of responses is equal or less than 1 the study will terminate at this stage. Under the hypothesis that P_0 is the true rate such a result has a probability of 0.72 to occur. If r_1 is greater than 1 then the enrollment will continue until the end of the second stage. The second stage ends when the 18th patient has been enrolled. The therapy will be considered a success if the number of responders is equal or greater than 6.

The parameters P_0 and P_1 have been chosen arbitrarily on the basis of what is reasonably expected, as so far, no study has been carried out on the effect of this therapy.

On the basis of the results obtained investigations might be made to find out the reasons why the therapy may or may not benefit some patients.

In order to conduct a biosimilarity evaluation between fresh cells vs frozen cells, an expansion of a cohort (3-6 treated patients) is required.

12.2 Evaluable patients

For Immune-reconstitution: Patients will be evaluable for assessment of immune-reconstitution if they have completed all 4 planned infusions of manipulated lymphocytes and have survived for at least 4 weeks after the fourth lymphocytes infusion. Additionally, any patient who fulfills the criteria for immune-reconstitution (see 6.1 and 10.2) at any given time after the first lymphocyte infusion, will also be evaluable for assessment.

For GvHD: Patients will be evaluable for GvHD if they have received at least one infusion of engineered lymphocytes and have survived for a minimum of 2 weeks. Additionally, any patient who develops GvHD after the infusion of any of the lymphocyte infusions will also be evaluable for assessment.

For efficacy of the control of GvHD: Patients will be evaluable for efficacy of the control of GvHD after administration of ganciclovir in patients treated with HSV-tk transduced cells, if they develop grade \geq II GvHD of any system and receive Ganciclovir for at least 3 days.

12.3 Criteria for evaluation of activity

The main outcomes of the study are:

- Incidence of immune reconstitution
- Incidence of GvHD and GvL effect
- Incidence of positive response after the administration of ganciclovir in patients experiencing GvHD

The outcomes related to the parameter “ immune-reconstitution “ will be observed over a period of a month every week after each potential infusion. Patients will be followed up over a period of 6 months after the last T cell infusion.

The incidence of immune-reconstitution will be calculated by dividing the number of patients who experience an immune-reconstitution (defined as number of circulating CD3+ \geq 100/ μ l for two consecutive observations) by the total number of patients evaluable. A patient will be considered a non responder if after 30 days from the last potential infusion he/she will not show any kind of immune reconstitution.

The number of lymphocytes CD3⁺, CD3⁺/CD4⁺ and CD3⁺/CD8⁺ will be reported graphically and described analytically in relation to time and doses.

The GvL effect will be evaluated according to standard criteria for specific disease

The incidence of GvHD will be calculated as rate, the number of patients developing GvHD divided by the total number of patients evaluable. An early stopping rule for GvHD is adopted.

The incidence of positive responses after the administration of gancyclovir in patients experiencing GvHD will be calculated as a rate, the number of patients who respond positively to gancyclovir divided by the total number of patients being treated with gancyclovir (as treatment for GvHD)

12.4 Criteria to evaluate the tolerability

The side effects reported during the study according to chapter 11.1 will be recorded using the CRF and SAE forms according to the criteria of intensity and correlation proposed by the National Cancer Institute (NCI-Common Toxicity Criteria).

These side effects have to be shown to be correlated with the treatments under study.

12.5 Criteria for exclusion criteria from the statistical analysis

The statistical analysis will be conducted using the intention-to treat analysis (ITT) and the standard analysis (SA). In the ITT analysis, all patients enrolled in the study will be evaluated for efficacy and tolerability analyses, whereas in the SA, only patients fulfilling the inclusion criteria and correctly following the procedures and the controls planned in the protocol will be considered in the analysis.

12.6 Stopping rules

An early stopping rule will be adopted in presence of:

IMMUNE-RECONSTITUTION: The study will continue to recruit patients until 7 evaluable patients for immune -reconstitution have been enrolled. At this point, the decision of whether or not to terminate after the first stage will be based on the number of responses observed for those 7 evaluable patients. The study will terminate at the end of the first stage if 1 or fewer responses are observed. If the responses are greater than 1 then the enrollment will continue until the end of the second stage. The second stage ends when the 18th evaluable patient has been enrolled. The therapy will be considered a success if the number of responders is equal to or greater than 6.

GvHD: The study will temporarily stop and the documentation referred to the safety committee if any of the following occur:

First infusion of HSV-tk transduced lymphocytes: After the first 7 patients evaluable for GvHD have received the first dose of HSV-tk transduced lymphocytes, the number of patients developing grade \geq II GvHD of any system and not responding to Ganciclovir or other immunosuppressive therapy is greater than 3.

The number of patients developing grade \geq II GvHD of any system and not responding to Ganciclovir or other immunosuppressive therapy is greater than 3 in any subsequent cohort of 7 evaluable patients receiving the first dose of HSV-tk transduced lymphocytes.

Second infusion of HSV-tk transduced lymphocytes: The number of patients developing grade \geq II GvHD of any system and not responding to Ganciclovir or other immunosuppressive therapy is greater than 3 in the first or any subsequent cohort of 7 patients evaluable for GvHD after receiving the second dose of HSV-tk transduced lymphocytes.

Third infusion of HSV-tk transduced lymphocytes: The number of patients developing grade \geq II GvHD of any system and not responding to Ganciclovir or other immunosuppressive therapy is greater than 3 in the first or any subsequent cohort of 7 patients evaluable for GvHD after receiving the third dose of HSV-tk transduced lymphocytes.

Fourth infusion of HSV-tk transduced lymphocytes: The number of patients developing grade \geq II GvHD of any system and not responding to Ganciclovir or other immunosuppressive therapy is greater than 3 in the first or any subsequent cohort of 7 patients evaluable for GvHD after receiving the third dose of HSV-tk transduced lymphocytes.

13 Ethics and general study administration

13.1 Ethical aspects

The investigator will ensure that this study is conducted in full conformity with the principles of the Declaration of Helsinki (as amended in Tokyo, Venice, Hong Kong and South Africa) or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual.

The study must fully adhere to the principles outlined in “ Guideline for Good Clinical Practice” ICH tripartite guideline (January 1997) or with local law if it affords greater protection to the subject.

13.2 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) as well as any advertising or compensation given to the patient, will be submitted by the investigator to an independent 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 investigator to the Committee in accordance with local procedures and regulatory requirements.

When no local review board exists, the investigator is expected to submit the protocol to a regional Committee. If no regional committee exists, MolMed will assist the investigator in submitting the protocol to the European review committee.

13.3 Informed consent

It is the 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 after adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study.

For subjects not qualified or incapable of giving legal consent, written consent must be obtained from the legally acceptable representative.

In the case where both the subject and his/her legally acceptable representative are unable to read, an impartial witness 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.

The investigator or designee must also explain that the subjects are completely free to refuse to enter the study or to withdraw from it at any time, for any reason. The case report form for this study contains a section for documenting informed subject consent and this must be completed appropriately.

If new safety information results in a significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary.

All subjects (including those already being treated) should be informed of the new information, given a copy of the revised form and give their consent to continue the study.

13.4 Conditions for modifying the protocol

Protocol modifications to ongoing studies must be made only after consultation between an appropriate representative of the sponsor and the investigator. Protocol modifications must be prepared by a representative of the sponsor and the investigator. Protocol modifications must be prepared by a representative of the sponsor and initially reviewed and approved by the Clinical Manager and Biostatistician.

All protocol modifications must be submitted to the appropriate independent Ethics Committee or Institutional Review Board for information and approval 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 aspects of the trial (change in monitor, change in telephone-fax number).

13.5 Conditions for terminating the study

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this 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 ensure that adequate consideration is given to the protection of the patient's interest.

13.6 Study documentation CRFs 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.

-investigator's study file

-subject clinical source documents.

The investigator's study file will contain the protocol/amendments CRF and query forms, IRB approvals with correspondence, sample informed consent, drug/product records, staff CV's, and authorization forms and other appropriate documents/correspondence.

Subject clinical source documents will include patient hospital/clinic records, physician's and nurse's 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 enrollment logs. The investigator must keep these two categories of documents on file for at least 15 years after completion or discontinuation of the study.

After that period of time the documents may be destroyed, subject to local regulations.

Should the investigator be unable to guarantee this archiving requirement at the investigational site for any or all the documents special arrangements must be made between the investigator and sponsor to store these in a sealed container outside of the site so that they can be returned sealed to the investigator in the event of a 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.

13.7 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. This is particularly important when case report forms are illegible or when errors in data transcription are suspected. 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 confidentiality is protected.

13.8 Audit and inspections

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

13.9 Case report form

For each patient enrolled a case report form (CRF) must be completed and signed by the principal investigator or authorized delegate from the study staff.

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. All forms should be typed or filled out using indelible ink and must be legible. Errors should be crossed out but not obliterated, the correction inserted and the change initialed and dated by the investigator.

The investigator should ensure the accuracy completeness legibility and timelines of the data reported to the sponsor in the CRFs and in all required reports.

13.10 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 accordance with local requirements.

It will be the monitor's responsibility to inspect the CRF 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 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.

13.11 Confidentiality of trial documents and subject records

The investigator must ensure 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. The investigator should keep a subject enrollment log showing code names and addresses. The investigator should maintain documents not for submission to MolMed (e.g. written informed consent) in strict confidence.

13.12 Publication of data and protection of trade secrets

The results of this study may be published or presented at scientific meetings. If this is foreseen the investigator agrees to discuss any 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 yet be available to the investigator.

In accordance with standard editorial and ethical practice MolMed will generally support publication of multicenter trials only in their entirety and not as individual center data. Authorship will be determined by mutual agreement.

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Appendix A: Clinical Stages of GvHD

| Stage | SKIN | LIVER | GUT |
|-------|--|------------------------|---------------------------------|
| 0 | No rash | Bilirubin < 2 mg/dl | Diarrhea < 500 ml/day |
| 1 | Maculopapular eruption involving <25% of body surface | Bilirubin = 2-3 mg/dl | Diarrhea 500-1000 ml/day |
| 2 | Maculopapular eruption involving 25-50% of body surface. | Bilirubin = 3-6 mg/dl | Diarrhea 1000-1500 ml/day |
| 3 | Generalized erythroderma. | Bilirubin = 6-15 mg/dl | Diarrhea > 1500 ml |
| 4 | Generalized erythroderma with desquamation and bullae | Bilirubin > 15 mg/dl | Diarrhea >2000 ml pain or ileus |

| GRADE* | Organ Stage (S=skin, G=gut, L=Liver) | Clinical performance |
|---------------------|--------------------------------------|----------------------|
| 0 none | S = 0, G = 0, L = 0 | No decrease |
| I mild | S = 1-2, G = 0, L = 0 | No decrease |
| II moderate | S = 1-3, G = 1, and/or L = 1 | Mild decrease |
| III severe | S = 2-3, G = 2-3, and/or L = 2-4 | Marked decrease |
| IV life threatening | S = 2-4, G = 2-4, and/or L = 2-4 | Extreme decrease |

| GRADE** | Maximum Organ Stage (S=skin, G=gut, L=Liver) | Index |
|---------|--|-------|
| 0 | S = 0, G = 0, L = 0 | 0 |
| 1 | S = 1, G = 0, L = 0 | A |
| 2 | S, G = 1, and/or L = 2; or G and/or L =1 | B |
| 3 | S, G and/or L = 3 | C |
| 4 | S, G and/or L = 4 | D |

* Gluckdberg grade of acute GvHD

** IBMTR GvHD Severity Index

| | | |
|---------------|--------------------------------|-------------------------|
| MolMed S.p.A. | CLINICAL STUDY PROTOCOL | Internal Code: IPR/01 G |
|---------------|--------------------------------|-------------------------|

Appendix B: Toxicity Criteria according to NCI

| | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---|---|--|--|---|--|
| HAEMATOLOGICAL (ADULTS) | | | | | |
| Haemoglobin | ≥ 11.0 g/100 ml ≥ 110 g/dl ≥ 6.8 mmol/l | 9.5-10.9 g/100 ml 95-109 g/dl 5.6-6.7 mmol/l | 8.0-9.4 g/100 ml 80-94 g/l 4.95-5.8 mmol/l | 6.5-7.9 g/100 ml 65-79 g/l 4.0-4.9 mmol/l | < 6.5 g/100 ml < 65 g/l < 4.0 mmol/l |
| Leukocytes (1000/mm³) | ≥ 4.0 | 3.0-3.9 | 2.0-2.9 | 1.0-1.9 | < 1.0 |
| Granulocytes (1000/mm³) | ≥ 2.0 | 1.5-1.9 | 1.0-1.4 | 0.5-0.9 | < 0.5 |
| Platelets (1000/mm³) | ≥ 100 | 75-99 | 50-74 | 25-49 | < 25 |
| Haemorrhage | None | Petechiae | Mild blood loss | Gross blood loss | Debilitating blood loss |
| GASTROINTESTINAL | | | | | |
| Bilirubin | ≤ 1.25 x N* | 1.26-2.5 x N* | 2.6-5 x N* | 5.1-10 x N* | > 10 x N* |
| Transaminases (SGOT SGPT) | ≤ 1.25 x N* | 1.26-2.5 x N* | 2.6-5 x N* | 5.1-10 x N* | > 10 x N* |
| Alkaline phosphatase | ≤ 1.25 x N* | 1.26-2.5 x N* | 2.6-5 x N* | 5.1-10 x N* | > 10 x N* |
| Oral | No change | Soreness/erythema | Erythema, ulcers : can eat solids | Ulcers : requires liquid diet only | Alimentation not possible |
| Nausea/vomiting | None | Nausea | Transient vomiting | Vomiting requiring therapy | Intractable vomiting |
| Diarrhoea | None | Transient < 2 days | Tolerable, but > 2 days | Intolerable, requiring therapy | Haemorrhagic, dehydration |
| RENAL | | | | | |
| Blood urea nitrogen or blood urea creatinine | ≤ 1.25 x N* | 1.26-2.5 x N* | 2.6-5 x N* | 5-10 x N* | > 10 x N* |
| Proteinuria | No change | 1 + < 0.3 g % < 3 g/l | 2-3 + 0.3-1.0 g % 3-10 g/l | 4 + > 1.0 g % > 10 g/l | Nephrotic syndrome |
| Haematuria | No change | Microscopic | Gross | Gross + clots | Obstructive uropathy |
| Pulmonary | No change | Mild symptoms | Exertional dyspnoea | Dyspnoea at rest | Complete bed rest required |

| | | |
|----------------------|--------------------------------|-------------------------|
| MolMed S.p.A. | CLINICAL STUDY PROTOCOL | Internal Code: IPR/01 G |
|----------------------|--------------------------------|-------------------------|

| | Grade 0 | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---------------------------------|----------------|---|---|---|---|
| PULMONARY | | | | | |
| Fever with drug | None | Fever < 38°C | Fever 38-40°C | Fever > 40°C | Fever with hypotension |
| Allergic | No change | Aedema | Bronchospasm : no parenteral therapy needed | Bronchospasm : parenteral therapy required | Anaphylaxis |
| CUTANEOUS - ANNEX | | | | | |
| Cutaneous | No change | Erythema | Dry desquamation, vesiculation, pruritus | Most desquamation, ulceration | Exfoliative dermatitis : necrosis requiring surgical intervention |
| Hair | No change | Minimal hair loss | Moderate, patchy alopecia | Complete alopecia but reversible | Non-reversible alopecia |
| Infection (specify site) | None | Minor infection | Moderate infection | Major infection | Major infection with hypotension |
| CARDIAC | | | | | |
| Rhythm | No change | Sinus tachycardia, > 110 at rest | Unifocal PVC, atrial arrhythmia | Multifocal PVC | Ventricular tachycardia |
| Function | No change | Asymptomatic, but abnormal cardiac sign | Transient symptomatic dysfunction : no therapy required | Symptomatic dysfunction responsive to therapy | Symptomatic dysfunction non-responsive to therapy |
| Pericarditis | No change | Asymptomatic effusion | Symptomatic : no tap required | Tamponade : tap required | Tamponade : surgery required |
| NEUROTOXICITY | | | | | |
| State of consciousness | Alert | Transient therapy | Somnolence <50% of waking hours | Somnolence >50% of waking hours | Coma |
| Peripheral | None | Paresthesias and or decreased tendon reflexes | Severe paresthesias and or mild weakness | Intolerable paresthesias and or marked motor loss | Paralysis |
| Constipation** | None | Mild | Moderate | Abdominal distension | Distension and vomiting |
| Pain+ | None | Mild | Moderate | Severe | Intractable |

N* : upper limit of normal value of population under study.

** This does not include constipation resultant from narcotics

+ Only treatment-related pain is considered, not disease-related pain.

Use of narcotics may be helpful in grading pain depending on the patient's tolerance.

Appendix C: Summary of the characteristics of the product (GANCICLOVIR)

TRADE NAME OF THE MEDICINAL PRODUCT

Cymevene® powder for infusion

QUALITATIVE AND QUANTITATIVE COMPOSITION

Ganciclovir 500mg (as ganciclovir sodium 546mg).

PHARMACEUTICAL FORM

Sterile, freeze-dried powder for reconstitution with Water for Injections.

CLINICAL PARTICULARS

Therapeutic indications

Cymevene is indicated for the treatment of life-threatening or sight-threatening cytomegalovirus (CMV) infections in immunocompromised individuals. These states include acquired immunodeficiency syndrome (AIDS), iatrogenic immunosuppression associated with organ transplantation, or chemotherapy for neoplasia.

Cymevene may also be used for the prevention of CMV disease, specifically in those patients receiving immunosuppressive therapy secondary to organ transplantation.

POSODOLOGY AND METHOD OF ADMINISTRATION

For intravenous infusion following reconstitution with 10ml Water for Injections BP. Based on patient weight and therapeutic indication the appropriate calculated dose volume should be removed from the vial (ganciclovir concentration 50mg/ml) and added to an acceptable infusion fluid (typically 100ml) for delivery over the course of 1 hour. Infusion concentrations greater than 10mg/ml are not recommended. (See section 6.6 *Instructions for use/handling*).

Adults

Treatment of CMV infection

Initial (induction) treatment: 5mg/kg infused at a constant rate over 1 hour every 12 hours (10mg/kg/day) for 14 to 21 days.

Long-term (maintenance) treatment: For immunocompromised patients at risk of relapse of CMV retinitis a course of maintenance therapy may be given. Intravenous infusion of 6mg/kg once daily 5 days per week, or 5mg/kg once daily 7 days per week is recommended.

Treatment of disease progression: Indefinite treatment may be required in patients with AIDS, but even with continued maintenance treatment, patients may have progression of retinitis. Any patient in whom the retinitis progresses, either while on maintenance treatment or because treatment with Cymevene has been withdrawn, may be re-treated using the induction treatment regimen.

Prevention of CMV disease

Induction regimen: 5mg/kg infused every 12 hours (10mg/kg/day) for 7 to 14 days.

Maintenance regimen: Intravenous infusion of 6mg/kg once daily 5 days per week, or 5mg/kg once daily 7 days per week is recommended.

Renal impairment

For patients with renal insufficiency the dose should be modified according to creatinine clearance, as shown in the table below:

Creatinine clearance can be related to serum creatinine by the following formulae:

$$\text{For males} = \frac{(140 - \text{age}[\text{years}]) \times (\text{body weight} [\text{kg}])}{(0.81) \times (\text{serum creatinine} [\mu\text{mol/l}])}$$

$$\text{For females} = 0.85 \times \text{male value}$$

| Creatinine Clearance | Induction dose |
|-----------------------------|-----------------------------------|
| ≥ 70ml/min | 5.0mg/kg q12h |
| 50 - 69ml/min | 2.5mg/kg q12h |
| 25 - 49ml/min | 2.5mg/kg/day |
| 10 - 24ml/min | 1.25mg/kg/day |
| < 10ml/min | 1.25mg/kg/day after haemodialysis |

Patients undergoing dialysis should be given 1.25mg/kg/24 hours. On days when dialysis is performed the dose should be given shortly after the dialysis session.

As dosage modifications are recommended in patients with renal impairment, serum creatinine or creatinine-clearance levels should be monitored carefully. (See also section 5.2 *Pharmacokinetics: Renal impairment*). The optimal maintenance dose for patients with renal insufficiency is not known.

Use in the elderly

No studies on the efficacy or safety of Cymevene in elderly patients have been conducted. Since elderly individuals often have reduced renal function, Cymevene should be administered to elderly patients with special consideration for their renal status.

Use in children

There has been limited clinical experience in treating patients under the age of 12 years (see section 5.2 *Pharmacokinetic properties*). Reported adverse events were similar to those seen in adults. However, the use of Cymevene in children warrants extreme caution due to the potential for long-term carcinogenicity and reproductive toxicity. The benefits of treatment should outweigh the risks. Cymevene is not indicated for the treatment of congenital or neonatal CMV infections.

Laboratory monitoring and dosage reduction

Granulocytopenia (neutropenia), anaemia, thrombocytopenia and leucopenia have been observed in patients treated with ganciclovir.

Regular clinical and haematological assessments are recommended. White blood cell and platelet counts should be performed every two days for the first 14 days of treatment. Patients with a history of marrow sensitivity to ganciclovir or other nucleoside analogues, or with white blood cell counts less than 1000 cells/ μ l at the beginning of treatment should be monitored daily. During maintenance treatment complete blood counts are recommended weekly, or more frequently if counts are low.

Severe neutropenia (< 500 cells/ μ l) requires a dose interruption. For less severe neutropenia or other cytopenias a reduction in the total daily dose should be considered. Cell counts usually normalise within 3 to 7 days after discontinuing the drug or decreasing the dose. As evidence of marrow recovery becomes apparent gradual increases in dose, with careful monitoring of white blood cell counts, may be appropriate.

Contra-indications

- i) Pregnancy and lactation.
- ii) Patients with known hypersensitivity to Cymevene or to acyclovir.

SPECIAL WARNINGS AND SPECIAL PRECAUTIONS FOR USE

In preclinical testing ganciclovir caused aspermatogenesis, mutagenicity, teratogenicity and carcinogenicity. It should therefore be considered a potential carcinogen and teratogen in humans.

The clinical toxicity of Cymevene includes neutropenia, anaemia and thrombocytopenia. Cymevene should be used with caution in those patients with a history of cytopenia, and Cymevene should not be administered if the absolute neutrophil count falls below 500 cells/ μ l or the platelet count is less than 25,000 cells/ μ l.

Because of the mutagenic potential of Cymevene, women of childbearing potential should be advised to use effective contraception during treatment. Likewise, men should be advised to practise barrier contraception during and for at least 90 days following treatment.

Administration of Cymevene by intravenous infusion should be accompanied by adequate hydration, since Cymevene is excreted by the kidneys and normal clearance depends upon adequate renal function. If renal function is impaired, dosage adjustments based on creatinine clearance are required. (see section 4.2 *Posology and method of administration*).

INTERACTION WITH OTHER MEDICAMENTS AND OTHER FORMS OF INTERACTION

Binding of ganciclovir to plasma proteins is only about 1 - 2% and drug interactions involving binding site displacement are not anticipated. It is possible that drugs which inhibit renal tubular secretion or resorption, may reduce renal clearance of ganciclovir and could increase the plasma half-life of ganciclovir. It is also possible that drugs which inhibit replication of rapidly dividing cell populations such as bone marrow, spermatogonia, and germinal layers of skin and gastrointestinal mucosa might have combined additive toxic effects when used concomitantly with, before, or after Cymevene. Because of the possibility of additive toxicity with co-administration of drugs such as dapsone, pentamidine, flucytosine, vincristine, vinblastine, adriamycin, amphotericin

B, trimethoprim/sulpha combinations or other nucleoside analogues, combination with Cymevene therapy should be used only if the potential benefits outweigh the risks.

Probenecid: May increase serum concentrations of ganciclovir.

Zidovudine: Patients with AIDS may be receiving, or have received, treatment with zidovudine. Since both zidovudine and Cymevene can result in neutropenia (granulocytopenia) and anaemia, it is recommended that these two drugs not be given concomitantly during induction treatment with ganciclovir. In addition, data from a small number of patients studied to date indicate that maintenance ganciclovir treatment plus zidovudine at the recommended dose resulted in severe neutropenia in most individuals. Patients should be monitored for these events.

Didanosine: Cymevene dosing may increase the AUC of didanosine (induction regimen by about 70%, maintenance regimen by about 50%). Patients should be closely monitored for didanosine-related adverse events.

Imipenem-cilastatin: Generalised seizures have been reported in patients who received ganciclovir and imipenem-cilastatin. These drugs should not be used concomitantly unless the potential benefits outweigh the risks.

Mycophenolate: Based on the results of a single dose administration study of recommended doses i.v. ganciclovir and oral mycophenolate and the known effects of renal impairment on the pharmacokinetics of ganciclovir (see section 5.2 *Pharmacokinetics* and section 4.2 *Posology and method of administration*) and mycophenolate, it is anticipated that co-administration of these agents (which compete for mechanisms of renal tubular secretion) will result in increases in ganciclovir concentration and MPAG (inactive metabolite of mycophenolate). In patients with renal impairment in which ganciclovir and mycophenolate are co-administered, the dose recommendations for ganciclovir should be observed and patients monitored carefully. No substantial alteration of MPA (active metabolite of mycophenolate) pharmacokinetics is anticipated and mycophenolate adjustment is not required.

PREGNANCY AND LACTATION

Teratogenicity has been observed in animal studies. Cymevene should not be given to pregnant women as there is a high likelihood of damage to the developing foetus.

Adverse effects were observed in the offspring of lactating animals. It is not known if ganciclovir is excreted in human milk. However, since many drugs are, Cymevene should not be given to lactating mothers. The minimum time interval before breastfeeding can safely be resumed after the last dose of Cymevene is unknown.

EFFECTS ON ABILITY TO DRIVE AND USE MACHINES

Seizures, somnolence, dizziness, ataxia, confusion and/or coma may occur in patients receiving Cymevene. If patients are found to suffer from these effects, they should be advised not to undertake activities requiring constant alertness such as driving or operating machinery.

UNDESIRABLE EFFECTS

The following adverse events can occur in patients treated with i.v. administered Cymevene. Some of these may be due to the underlying disease. In three controlled clinical trials, the frequency of

the adverse events, which were thought to be probably or possibly related to treatment with Cymevene Powder for Infusion was less than 1% (< 1%) unless otherwise indicated in parentheses.

Body as a whole: Abdomen enlarged, anorexia (< 5%), asthenia (<10%), cellulitis, chest pain, chills, fever, headache (< 5%), infections (< 5%), malaise, aedema, pain, photosensitivity reaction, sepsis (< 5%).

Cardiovascular system: Arrhythmias, deep thrombophlebitis, hypertension, hypotension, migraine, phlebitis (< 5%), vasodilation.

Central nervous system: Abnormal gait, abnormal thoughts or dreams, anxiety, ataxia, coma, confusion, depression, dizziness, dry mouth, euphoria, hypoesthesia, insomnia, manic reaction, nervousness, paraesthesia (< 5%), psychosis, seizures, somnolence, tremor.

Gastro-intestinal system: Abdominal pain (< 5%), constipation, diarrhoea (< 10%), dyspepsia, dysphagia, eructation, faecal incontinence, flatulence (< 5%), haemorrhage, liver function test abnormality (< 5%), mouth ulceration, nausea (< 10%), pancreatitis, vomiting (< 5%).

Haemopoietic and lymphatic system: Anaemia (< 15%), eosinophilia, hypochromic anaemia, leucopenia (< 40%), marrow depression, pancytopenia, thrombocytopenia (< 5%).

Injection site: Abscess, inflammation (< 5%), pain (< 5%), phlebitis.

Metabolic and nutritional disorders: Alkaline phosphatase increased, creatinine increased, creatine phosphokinase increased, decreased blood sugar, hypokalaemia, lactic dehydrogenase increased, SGOT increased, SGPT increased.

Musculoskeletal system: Myalgia, myasthenia.

Respiratory system: Dyspnoea, increased cough.

Skin and appendages: Acne, alopecia (< 5%), herpes simplex, maculopapular rash, pruritus, rash (< 5%), sweating, urticaria.

Special senses: Abnormal vision, amblyopia, blindness, conjunctivitis, deafness, eye pain, retinal detachment, retinitis, taste perversion, vitreous disorder.

Urogenital system: Abnormal kidney function, breast pain, creatinine clearance decrease, haematuria, increased blood urea nitrogen (BUN), kidney failure, urinary frequency, urinary tract infection.

OVERDOSE

Adverse events following overdosage with Cymevene i.v. solution include:

Haematological toxicity: Irreversible pancytopenia, reversible leucopenia, persistent bone marrow suppression, reversible neutropenia or granulocytopenia.

Hepatotoxicity: Hepatitis.

Renal toxicity: Worsening of haematuria, elevated creatinine.

Gastrointestinal symptoms: Diarrhoea and/or vomiting.

Neurotoxicity: Generalised tremor and seizure.

In the event of an overdose, dialysis and hydration may be of benefit in reducing drug plasma levels.

PHARMACOLOGICAL PROPERTIES - PHARMACODYNAMIC PROPERTIES

Ganciclovir is a synthetic nucleoside analogue of 2'-deoxyguanosine which inhibits replication of herpes viruses both *in vitro* and *in vivo*. Sensitive human viruses include cytomegalovirus (CMV), herpes simplex virus-1 and -2 (HSV-1 and HSV-2), Epstein-Barr virus (EBV) and varicella zoster virus (VZV). Clinical studies have been limited to assessment of efficacy in patients with CMV infection.

In infected cells ganciclovir is initially phosphorylated to ganciclovir monophosphate. Further phosphorylation to ganciclovir triphosphate occurs by several cellular kinases. In CMV-infected cells there are higher levels of both cellular kinases and ganciclovir triphosphate as compared to non-infected cells. Thus, there is a preferential phosphorylation of ganciclovir in virus-infected cells. In virus-infected cells ganciclovir triphosphate is metabolised slowly, with 60 to 70% remaining intracellularly 18 hours after removal of ganciclovir from the extracellular fluid.

The antiviral activity of ganciclovir is the result of inhibition of viral DNA synthesis by: (1) competitive inhibition of incorporation of deoxyguanosine triphosphate into DNA by DNA polymerase and (2) incorporation of ganciclovir triphosphate into viral DNA causing termination of, or very limited, viral DNA elongation.

The possibility of viral resistance should be considered in patients who demonstrate poor clinical response or persistent viral excretion during therapy. Viral resistance has also been observed in patients receiving prolonged treatment for CMV retinitis with Cymevene.

PHARMACOKINETIC PROPERTIES

Absorption

At the end of a 1-hour i.v. infusion of 5mg/kg ganciclovir, total AUC ranged between 22.1 ± 3.2 (n = 16) and $26.8 \pm 6.1 \mu\text{g hr/ml}$ (n = 16) and C_{max} ranged between 8.27 ± 1.02 (n = 16) and $9.0 \pm 1.4 \mu\text{g/ml}$ (n = 16).

Distribution

The steady-state volume of distribution of ganciclovir after i.v. administration was $0.72 \pm 0.15 \text{ l/kg}$ (n = 66). Cerebrospinal fluid concentrations obtained 0.25 - 5.67 hours post-dose in 3 patients who received 2.5mg/kg ganciclovir i.v. q8h or q12h ranged from 0.31 - $0.68 \mu\text{g/ml}$ representing 24 - 70% of the respective plasma concentrations. Binding to plasma proteins was 1 - 2% over ganciclovir concentrations of 0.5 and $51 \mu\text{g/ml}$.

Metabolism and elimination

When administered i.v., ganciclovir exhibits linear pharmacokinetics over the range of 1.6 - 5.0mg/kg. Renal excretion of unchanged drug by glomerular filtration and active tubular secretion is the major route of elimination of ganciclovir. Systemic clearance of i.v. administered ganciclovir was $3.51 \pm 0.85 \text{ ml/min/kg}$ (n = 66) while renal clearance was $3.20 \pm 0.79 \text{ ml/min/kg}$ (n = 48). Half-life was 3.56 ± 1.06 hours (n = 66) following i.v. administration.

Pharmacokinetics in special clinical situations

Renal impairment leads to altered kinetics of ganciclovir as indicated below.

| Ganciclovir | | |
|--------------------------------------|--|---------------------------------|
| Serum creatinine (micromol/l) | Systemic plasma clearance (ml/min/kg) | Plasma half-life (hours) |
| < 124 (n = 22) | 3.64 | 2.9 |
| 125 - 225 (n = 9) | 2.00 | 5.3 |
| 226 - 398 (n = 3) | 1.11 | 9.7 |
| > 398 (n = 5) | 0.33 | 28.5 |

Haemodialysis reduces plasma concentrations of ganciclovir by about 50% after both i.v. and oral administration.

Children

Ganciclovir pharmacokinetics were also studied in 10 children, aged 9 months to 12 years. The pharmacokinetic characteristics of ganciclovir are similar after single and multiple (q12h) i.v. doses (5mg/kg). After the administration of a 5mg/kg single dose, the steady-state volume of distribution reported was 0.68 ± 0.20 l/kg, C_{max} was 7.59 ± 3.21 µg/ml, systemic clearance was 4.66 ± 1.72 ml/min/kg, and $t_{1/2}$ was 2.49 ± 0.57 hours. The pharmacokinetics of i.v. ganciclovir in neonates and children are similar to those observed in adults.

Elderly

No studies have been conducted in adults older than 65 years of age.

PRECLINICAL SAFETY DATA

From fertility studies in mice it is considered likely that ganciclovir causes temporary or permanent inhibition of spermatogenesis. Data from mice also indicate that suppression of fertility in females may occur.

PHARMACEUTICAL PARTICULARS

LIST OF EXCIPIENTS

None.

INCOMPATIBILITIES

The dry powder should not be reconstituted with bacteriostatic water containing parabens, since these are incompatible with ganciclovir sterile powder and may cause precipitation.

SHELF LIFE

36 months.

SPECIAL PRECAUTIONS FOR STORAGE

Undiluted vials: Do not store above 30°C.

From a microbiological point of view, the product should be used immediately after reconstitution and dilution. If the product is not used immediately, the in-use storage times and conditions prior to use are the responsibility of the user. Following reconstitution and dilution, the following in-use

storage times should be followed unless reconstitution and dilution has taken place in controlled and validated aseptic conditions.

In-use storage times for the reconstituted vial should not be longer than 12 hours. Do not refrigerate.

In-use storage time for the infusion solution should not be longer than 24 hours when stored in a refrigerator at 2 - 8°C. Freezing is not recommended.

NATURE AND CONTENTS OF CONTAINER

10ml multidose vials (type I, clear glass) with a grey butyl siliconised stopper in quantities of 5 or 25 vials.

INSTRUCTIONS FOR USE/HANDLING

Caution should be exercised in the handling of Cymevene. As Cymevene has shown carcinogenic and mutagenic activity, caution should be exercised in its handling. Avoid inhalation or direct contact of the powder contained in Cymevene vials or direct contact of the reconstituted solution with the skin or mucous membranes. Cymevene solutions are alkaline (pH approximately 11). If Cymevene contacts the skin or mucous membranes, wash thoroughly with soap and water. For eye exposure rinse thoroughly with plain water.

Reconstitution of the vial:

The contents of the vial should be reconstituted by the addition of 10ml Water for Injections BP. Do not use bacteriostatic water for injection containing parabens (para-hydroxybenzoates), since these are incompatible with ganciclovir and may cause precipitation.

The vial should be shaken to dissolve the drug. Typically, reconstitution takes less than one minute, although occasionally for some batches it may take up to 3 minutes.

Reconstituted solution should be inspected for particulate matter prior to proceeding with the admixture preparation. If there is any present, the drug should not be administered.

Reconstituted solution in the vial is stable at room temperature for 12 hours. It should not be refrigerated.

Infusion solution preparation and administration: Based on patient weight, the appropriate calculated dose volume should be removed from the vial (ganciclovir concentration 50mg/ml) and added to 100ml of a suitable infusion fluid for delivery, over the course of one hour. Infusion concentrations greater than 10mg/ml are not recommended. The following infusion fluids are compatible with ganciclovir: Sodium Chloride Intravenous Infusion BP (0.9% w/v); Glucose Intravenous Infusion BP (5% w/v); Compound Sodium Lactate Intravenous Infusion BP; Ringer's Solution for Injection. Cymevene should not be mixed with other i.v. products.

Because non-bacteriostatic infusion fluid must be used with Cymevene, the infusion solution must be used as soon as possible and within 24 hours of dilution to reduce the risk of bacterial contamination. The infusion solution should be refrigerated. Freezing is not recommended.

Method of administration: Cymevene must only be given by intravenous infusion, preferably via a plastic cannula, into a vein with adequate blood flow. Infusions are recommended to be given over at least one hour, since rapid or bolus intravenous injections may increase the toxicity. Intramuscular or subcutaneous injection may result in severe tissue irritation due to the high pH of the solution.

The recommended dosage, frequency, or infusion rates should not be exceeded,

MARKETING AUTHORISATION HOLDER

Roche Products Limited, 40 Broadwater Road, Welwyn Garden City, Hertfordshire, AL7 3AY.

MARKETING AUTHORISATION NUMBER

PL 0031/0465

DATE OF FIRST AUTHORISATION/RENEWAL OF AUTHORISATION

June 1998

DATE OF (PARTIAL) REVISION OF THE TEXT

October 2001

Appendix D: Summary of the characteristics of the product (INTERLEUKIN 2)

NAME OF THE MEDICINAL PRODUCT

Proleukin 18 x 10⁶ IU iv

QUALITATIVE AND QUANTITATIVE COMPOSITION

Proleukin powder for parenteral use contains aldesleukin a protein with a molecular weight of approximately 15600 daltons. It is produced by recombinant DNA technology using an E.coli strain which contains a genetically engineered modification of the human IL-2 gene. This modified recombinant human IL-2 differs from native IL-2 in the following ways:

the molecule is not glycosylated because it is derived from E. coli

the molecule has no N- terminal alanine

the molecule has serine substituted for cysteine at amino acid position 125

The two amino acid changes result in a more homogeneous IL-2 product. The biological activities of aldesleukin and native human IL 2 a natural lymphokine, are similar; both regulate the immune response.

When reconstituted with 1.2 ml sterile water for injection USP each vial delivers 1 ml solution containing 18 million IU aldesleukin, 50 mg mannitol, 0.2 mg sodium dodecyl sulphate, buffered with sodium phosphates to a pH of 7.5 (range 7.2 to 7.8).

PHARMACEUTICAL FORM

Proleukin is supplied as sterile white lyophilized powder for parental use.

CLINICAL PARTICULARS

Therapeutic indications

Treatment of metastatic renal cell carcinoma

Risk factors associated with decrease response rates and median survival are:

a performance status of ECOG 1 or greater

more than one organ with metastatic disease sites

a period of < 24 months between initial diagnosis of primary tumor and the date the patient is evaluated for proleukin treatment.

Response rates and median survival decrease with the number of risk factors present. Patients positive for all the three risk factors should not be treated with Proleukin.

POSODOLOGY AND METHOD OF ADMINISTRATION

Before initiating treatment carefully review the CONTRAINDICATIONS and SPECIAL WARNINGS AND PRECAUTIONS FOR USE sections.

Proleukin should be administered intravenously by continuous infusion. The following dosage regimen is recommended to treat adult patients with metastatic renal cell carcinoma.

18 x 10⁶ IU per sqm per 24 hours as a continuous infusion for 5 days followed by 2-6 days without drug an additional 5 days iv. Proleukin as continuous infusion and 3 weeks without drug. This

constitutes one induction cycle. After the 3 week rest period of the first cycle, a second induction cycle should be given.

Up to 4 maintenance cycles (18×10^6 IU per sqm as continuous infusion for 5 days) may be given with 4 week interval to patients who respond or have disease stabilization.

If a patient cannot tolerate the recommended dosage regimen, the dose should be reduced or the administration interrupted until the toxicity has moderated. It is not known to what extent dose reduction affects response rates and median survival.

Elderly: elderly patients may be more susceptible to the side effects of Proleukin and caution is recommended in the treatment of such patients.

Children: safety and efficacy of Proleukin in children have not yet been established.

Contraindications

Proleukin therapy is contra-indicated in the following patients:

patients with a performance status of ECOG ≥ 2

patients with a simultaneous presence of a performance status of ECOG 1 or greater and more than one organ with metastatic disease sites and a period of < 24 months between initial diagnosis of primary tumour and the date the patient is evaluated for Proleukin treatment

patients with a significant history or current evidence of severe cardiac disease. In questionable cases a stress test should be performed

patients with evidence of active infection requiring antibiotic therapy

patients with a $pO_2 < 60$ mmHg during rest

patients with pre-existing severe major organ dysfunction

patients with CNS metastases or seizure disorders, with the exception of patient with successfully treated brain metastasis (negative CT; neurologically stable)

patients with a known history of hypersensitivity to human recombinant interleukin 2

In addition it is recommended to exclude the following patients:

patients with $WBC < 4.000/mm^3$, platelets $< 100.000/mm^3$; HCT $< 30\%$

patients with serum bilirubin and creatinine outside normal range

patients with organ allografts

patients who are likely to require corticosteroids

patients with pre-existing auto-immune disease

SPECIAL WARNINGS AND PRECAUTIONS FOR USE

Patient screening

See also contraindications section. Clinical studies have shown that patients with metastatic renal cell carcinoma can be divided into 4 distinct risk groups predictive for survival and to some extent response following Proleukin therapy. The 4 risk groups are defined by the number of risk factors present at treatment start: the very low risk group has no risk factor, the low risk group one risk factor, the median group any combination of 2 risk factors and the high risk group has the simultaneous presence of all 3 risk factors.

Response rates and median survival decrease with the number of risk factors present.

Patient positive for all three risk factors should not be treated with Proleukin.

Risk factors are the following:

ECOG baseline performance status ECOG 1 or higher

Time from diagnosis of primary tumour to Proleukin therapy < 24 months
≥ 2 metastatic sites where lung metastases bone metastases or other metastases are counted as single metastatic site.

Proleukin should only be used under the supervision of a qualified physician, experienced in the use of cancer chemotherapeutic agents. It is recommended that patients are admitted to a specialized unit having the facilities of an intensive care unit for monitoring the patient's relevant clinical and laboratory parameters.

Should serious adverse events occur, dosage should be modified according to the section "POSODOLOGY and METHOD OF ADMINISTRATION". It is important to note that adverse events, although sometimes serious or even life-threatening, are manageable and usually, although not invariably, resolve within 1 or 2 days of cessation of Proleukin therapy. The decision to resume therapy should be based on the severity and spectrum of the clinical toxicity.

Proleukin administration results in fever and gastrointestinal side effects in most patients treated at the recommended dose. Concomitant therapy with paracetamol can be instituted at the time of Proleukin administration to reduce fever. Pethidine may be added to control the rigours associated with fever. Antiemetics and antidiarrhoeals may be used as needed to treat other gastrointestinal side effects. Some patients with pruritic rash benefit from concomitant administration of antihistamines.

Proleukin administration results in reversible elevation of hepatic transaminases, serum bilirubin, serum urea and serum creatinine. Patients with pre-existing renal or hepatic dysfunction should be closely monitored. Renal or hepatic metabolism or excretion of concomitantly administered drugs may be altered by the administration of Proleukin. Other drugs with known nephrotoxic or hepatotoxic potential should be used with caution.

A capillary leak syndrome with hypotension is frequently reported; this usually begins within hours after initiation of Proleukin infusion.

In some patients hypotension resolved without therapy.

In others treatment is required with cautious use of iv fluids, albumin or in more refractory cases, low dose dopamine. If these measures are not successful, the Proleukin therapy should be interrupted.

If iv fluids are administered, care must be taken to weigh potential benefits of the expansion of intravascular volume against the risk of pulmonary oedema secondary to capillary leakage. Fluid and electrolyte balance should be monitored in all patients because Proleukin may cause renal dysfunction with oliguria.

In addition Proleukin may exacerbate effusions from serosal surfaces. Consideration should be given to treating these prior to initiation of Proleukin therapy, particularly when effusions are located in anatomic sites where worsening may lead to impairment of major organ function (e.g. pericardial effusions).

Pulmonary function should be monitored closely in patients who develop rales or increased respiratory rate, or who complain of dyspnea. Some patients may require intubation for management of transient respiratory failure.

Patients may experience mental status changes including irritability, confusion, or depression while receiving Proleukin. Although generally reversible when drug administration is discontinued, these

mental status changes may persist for several days. Proleukin may alter patient response to psychotropic drugs.

Proleukin administration should be discontinued in patients developing severe lethargy or somnolence; continued administration may result in coma.

Proleukin may exacerbate disease symptoms in patients with clinically unrecognized or untreated CNS metastases. All patients should have adequate evaluation and treatment of CNS metastases prior to receiving Proleukin therapy.

Proleukin may exacerbate pre-existing autoimmune disease, resulting in life-threatening complications. Because not all patients who develop interleukin 2 associated autoimmune phenomena have a pre existing history of autoimmune disease, awareness and close monitoring for thyroid abnormalities or other potentially autoimmune phenomena is warranted. A few patients with quiescent Crohn's disease had activation of their disease following treatment with Proleukin requiring surgical intervention.

Pre existing bacterial infections should be treated prior to initiation of Proleukin therapy. Toxicities associated with Proleukin administration may be exacerbated by concurrent bacterial infection.

Administration of Proleukin may be associated with an increased incidence and or severity of bacterial infection, including septicaemia, bacterial endocarditis, septic thrombophlebitis, peritonitis, pneumonia and local catheter site infection. Except for several cases due to Escherichia coli, causative organisms have been treated prophylactically with antibiotics.

Proleukin administration may cause anaemia and thrombocytopenia. Consequently, all patients should be monitored during treatment for hematological effects.

Laboratory and clinical tests: in addition to those tests normally required for monitoring patients with metastatic renal cell carcinoma, the following tests are recommended for all patients on Proleukin therapy prior to beginning treatment and then periodically thereafter:

Standard hematological tests , WBC (including differential and platelet counts)
Blood chemistry including electrolytes, renal and hepatic function tests
Chest X-rays

Baseline ECG (+ stress test if indicated) performance status, vital signs, objective evaluation for coronary vascular disease and in patient with a history of smoking or respiratory disease, pulmonary function tests with arterial blood gases are recommended as adjuncts to history and physical examination in the pre
Treatment evaluation of patients.

INTERACTION WITH OTHER MEDICAMENTS AND OTHER FORMS OF INTERACTION

Proleukin may affect central nervous function. Therefore interactions could occur following concomitant administration of centrally acting drugs.

Concurrent administration of drugs with hepato, nephro, mielo or cardiotoxicity may increase the toxicity of Proleukin in these systems.

Concomitantly administered glucocorticoids may decrease the activity of Proleukin. However, patients who develop life-threatening signs or symptoms may be treated with dexamethasone until toxicity resolves to an acceptable level.

Antihypertensive agents, such as beta blockers, may potentiate the hypotension seen with Proleukin. Use of contrast media after Proleukin administration may result in a recall of the toxicity observed during Proleukin administration. Most events were reported to occur within 2 weeks after the last dose of Proleukin but some occurred months later.

PREGNANCY AND LACTATION

Proleukin should not administered to fertile persons of either sex not practicing effective contraception. The safety of this medicinal product for use in human pregnancy has not been established. Experimental animal studies are insufficient to assess the safety with respect to reproduction development of the embryo, or fetus the course of gestation and peri post natal development. No information is available on the excretion of aldesleukin in human milk, or its effect on lactation and Proleukin should therefore not to be administered to lactating mothers.

EFFECTS ON ABILITY TO DRIVE AND USE MACHINES

Proleukin produces adverse events which affect the ability to drive and use machines. Patients are hospitalized during treatment; possible adverse effects will have disappeared by the time patients are dismissed from the hospital.

UNDESIRABLE EFFECTS

Frequency and severity of adverse reactions to Proleukin have generally been shown to be dependent on dose and schedule. Most adverse reactions are self limited and might reverse within 1 to 2 days of discontinuation of therapy. A small number of patients (3%) died of treatment related side effects.

Cardiovascular System

Frequently reported: mild to severe hypotension.

Occasionally reported: mild to severe arrhythmia, mild to severe tachycardia, angina pectoris.

Rarely reported: thrombosis, hypertension, palpitations, transient ECG changes, myocardial infarction, pulmonary embolism, phlebitis.

Kidneys

Frequently reported: Mild to severe oliguria with elevated serum urea and serum creatinine.

Rarely reported: hematuria

Respiratory Tract

Frequently reported: Mild to severe dyspnoea.

Occasionally reported: mild to severe pulmonary oedema.

Rarely reported: adult respiratory distress syndrome, mild to severe cyanosis, hypoxia, respiratory tract infection, mild to severe pleural effusion

Liver

Frequently reported: Mild to severe hyperbilirubinemia, mild to severe elevation of hepatic transaminases and alkaline phosphatases.

Gastrointestinal Tract

Frequently reported: Mild to severe nausea with or without vomiting, mild to moderate diarrhoea, mild to severe anorexia.

Rarely reported: dysphagia, rectal hemorrhage, dyspepsia, gastritis, constipation.

Blood

Frequently reported: Mild to severe anemia.

Note: during treatment most patients experience lymphocytopenia and eosinophilia with a rebound lymphocytosis within 24-48 hours following treatment. These are not considered adverse events and may be related to the mechanism of antitumour activity of Proleukin.

Occasionally reported: mild to severe thrombocytopenia, mild to moderate leukopenia, moderate coagulation disorders.

Rarely reported: epistaxis.

Nervous system

Occasionally reported: moderate to severe agitation/anxiety, mild to severe confusion dizziness, mild to severe somnolence, mild to severe central or peripheral motor neurological disorders. Rarely reported: Paraesthesia, syncope, depression, hallucinations, paralysis, speech disorders, convulsions.

Abnormal laboratory findings

Rarely reported: hypo or hyperthyroidism, hyperglycemia, hypo-hyperkalemia.

Skin and mucous membranes

Frequently reported: mild to severe erythema and rash.

Occasionally reported: mild to moderate conjunctivitis, mild to moderate mucositis, mild to severe pruritus, mild to severe skin exfoliation, mild to severe vitiligo.

Rarely reported: alopecia, nasal congestion.

Other side effects

Frequently reported: mild to moderate weight gain with oedema, mild to severe fever with or without chills, mild to severe malaise and fatigue.

Occasionally reported: moderate to severe pain, mild to severe headache.

Rarely reported: moderate to severe arthralgia, myalgia, ascitis.

OVERDOSE

Side effects following the use of Proleukin are dose related.

Therefore patients can be expected to experience these events in an exaggerated fashion when the recommended dose is exceeded.

Adverse reactions generally will reverse when the drug is stopped. Any continuing symptoms should be treated supportively.

PHARMACOLOGICAL PROPERTIES- PHARMACODYNAMIC PROPERTIES

Proleukin acts as a regulator of the immune response.

The administration of aldesleukin in murine tumor models has been shown to reduce both tumor growth and spread. The exact mechanism by which aldesleukin mediated immunostimulation leads to antitumor activity is not yet known.

PHARMACOKINETIC PROPERTIES

The serum half-life curves of aldesleukin in humans following iv bolus administration can be described as bi-exponential. The half life alfa is 13 minutes and the half life beta is 85 minutes. The alfa phase accounts for clearance of 87% of a bolus injection. Observed serum levels are proportional to the dose of aldesleukin.

The kidney is the major clearance route of rIL-2 in animals and most of the injected dose is metabolized in the kidney with no biologically active aldesleukin appearing in the urine.

The observed clearance rates in humans after short iv infusion (15 minutes) and after 24 hours continuous iv infusion approximate renal glomerular filtration clearance.

PRECLINICAL SAFETY DATA

None

PHARMACEUTICALS PARTICULARS**LIST OF EXCIPIENTS**

Proleukin contains mannitol, sodium dodecyl sulphate and sodium phosphate buffer.

INCOMPATIBILITES

Reconstitution and dilution procedures other than those recommended may result in incomplete delivery of bioactivity and or formation of biologically inactive protein.

Use of bacteriostatic water for injection or sodium chloride injection 0.9% should be avoided because of increased aggregation. Proleukin should not be mixed with other drugs.

It is recommended that devices or administration sets containing in-line filters are not used for delivery of Proleukin. Bioassays have shown significant loss of aldesleukin when filters are used.

SHELF LIFE

Do not use vials of lyophilized Proleukin beyond the expiration date marked on the vial label. Reconstituted Proleukin may be stored up to 24 hours. Diluted Proleukin should be used within 24 hours from the reconstitution. Note: this product contains no microbial preservative.

SPECIAL PRECAUTIONS FOR STORAGE

Store vials of lyophilized Proleukin in a refrigerator at 2° -8°. Reconstituted or diluted Proleukin may be stored at refrigerated and a room temperature (2 to 30° C).

NATURE AND CONTENTS OF CONTAINER

Proleukin is supplied in 5 ml rubber stoppered single use clear glass vials deliver 1 ml solution containing 18 x 10⁶ IU aldesleukin following reconstitution according to instructions.

INSTRUCTIONS FOR USE/HANDLING

Reconstitution Directions: each vial of Proleukin should be reconstituted with 1.2 ml of Sterile water for injection. Direct the diluent against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. The resulting solution should be a clear colourless liquid, containing 18 million IU aldesleukin per ml.

Dilution Directions: the total daily dose of reconstituted aldesleukin should be diluted as necessary to up to 500 ml of 5% Dextrose Injection containing 0.1 % human albumin and infused over a 24 hour period. Order of addition: Human albumin should be added and mixed with dextrose injection prior to addition of the reconstituted aldesleukin. Human albumin is added to protect against loss of bioactivity.

Proleukin contains no preservatives. It is therefore essential that the infusion solution is prepared using aseptic techniques.

Name or style and permanent address or registered place of business of the holder of the marketing authorization

CHIRON BV
Paasheuvelweg 30
1105 BJ Amsterdam
The Netherlands

Registered in NL under RVG 13354
Approved 3 February 1997

Appendix E: List of centers

- ❑ Department of Clinical and Experimental Medicine, Section of Haematology and Clinical Immunology, University of Perugia, Italy
- ❑ Department of Hematology, Faculty of Medicine, Imperial College, Hammersmith Hospital London, UK
- ❑ Department of Bone Marrow Transplantation & Cancer Immunotherapy, Hadassah University Hospital Jerusalem, Israel
- ❑ Bone Marrow Transplantation Unit, Istituto Scientifico San Raffaele, Milan, Italy
- ❑ Department of Medical Oncology and Hematology, Istituto Clinico Humanitas, Rozzano, Milan, Italy

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| MolMed S.p.A. | CLINICAL STUDY PROTOCOL | Internal Code: IPR/01 G |
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Appendix F: Supportive care

The recommended supportive care for this protocol is:

| | |
|---------------------|---|
| Bacteria | Ciprofloxacin 1000mg p.o. from -10 until neutropenia >500/ μ l. |
| | CEFTAZIDIME 100 MG/KG IV STARTING WHEN NEUTROPHILS B NEUTROPHILS ARE ABOVE 1000 /μL |
| | TMP/SMX 320/1600MG P.O. DAILY FROM -10 TO -2 BIWEEKLY FROM +30 TO +120. |
| Virus | Gancyclovir 10 mg/kg from -8 to -2 i.v. |
| | Acyclovir 0.5 gr/m ² x 3 from -1 to +4 i.v. |
| | Foscarnet 90 mg/kg from +5 to +21 i.v. |
| | Acyclovir 30 mg/kg from +22 to +180 p.o. |
| Fungi | Anfotericin B 1 mg/kg from -8 to +30 (liposomal) i.v. |
| | Itraconazole oral sol 400 mg from +31 to +120 p.o. |
| Mycobacteria | Azithromycin 1200 mg weekly from +20 to +90 p.o. |

Anti CMV prophylaxis: twice weekly monitoring of CMV antigenemia for preemptive therapy with gancyclovir will be performed until at least 6 weeks from transplant and before T cells infusion. High titer anti-CMV immunoglobulins 100 mg/Kg will be administered weekly until day 90.

Appendix G: Amendment history

IPR/01.B (23 October 2002)

Collection of Buffy coat screening phase

IPR/01.C (24 April 2003)

Evaluation of gene marking and immunophenotypes (LNGFR) at each local site

Modification of schedule for immunophenotypes

Usage of ISHAGE methods for CD3+, CD4+ and CD8+ evaluation

Inclusion of a specific chapter regarding a potential pregnancy during study

Possibility of DLI for disease relapse also after the 6th month of follow-up

IPR/01D (1 September 2003)

Clarification of safety data collection in case of DLI during the entire study and introduction of follow up phase for patients undergoing DLI for disease relapse

IPR/01E (1 March 2005)

Indication of reduced toxicity conditioning regimens for a subset of patients affected by lymphoid or myeloid haematological disease, with age >55 and/or presenting with associated comorbidity or organ impairment

IPR/01F (31 May 2005)

Introduction of a freezing step of the HSV-TK transduced lymphocytes prior to infusion.

The evaluation of biosimilarity between fresh cells vs frozen cells will be conducted on a cohort of 3-6 patients after the enrollment of 18 evaluable patients.

IPR/01G (14 June 2007)

Synopsis of the protocol page 2

Number of patient

Previous version

18 evaluable

Next version

30 evaluable

Synopsis of the protocol page 2

Study procedures

Previous version

Infusion of 10^6 CD3+ c/kg genetically modified at day+42

New version

Infusion of 1×10^7 CD3+ c/kg (or alternatively 1×10^6 CD3+ c/kg at Investigator discretion based on estimated infectious risk) genetically modified between day +21 and day +49 after SCT

Previous version

Day 72- 1×10^7 CD3+ c/kg

New version

30 days after 1st infusion

Previous version

Day 102- 1×10^6 CD3+ c/kg +IL2 (6.000.000 IU/m² sc x 5 days)

New version

30 days after 2nd infusion

Previous version

Day 132- 1×10^7 CD3+ c/kg +IL2 (6.000.000 IU/m² sc x 5 days)

New version

30 days after 3rd infusion

Genetically Modified Lymphocytes page 11**Previous version**

[omissis]

Lymphocytoapheresis will be obtained from the donor ($\geq 10^{10}$ mononuclear cells) as well as plasmapheresis (600 ml). The donor cells will then be frozen and for each dose planned will be thawed and stimulated with anti-CD3 and IL2. The transduction process will be performed by using the supernatant with retroviral vectors. The transduced cells which express LNGFR will be selected with a specific antibody and immunomagnetic beads.

Next version

[omissis]

Lymphocytoapheresis will be obtained from the donor ($\geq 10^{10}$ mononuclear cells) as well as plasmapheresis (600 ml plasma) and sent to MolMed as Fresh cells.

The donor cells will then be transduced by using the supernatant with retroviral vectors.

The transduced cells which express LNGFR will be selected with a specific antibody and immunomagnetic beads.

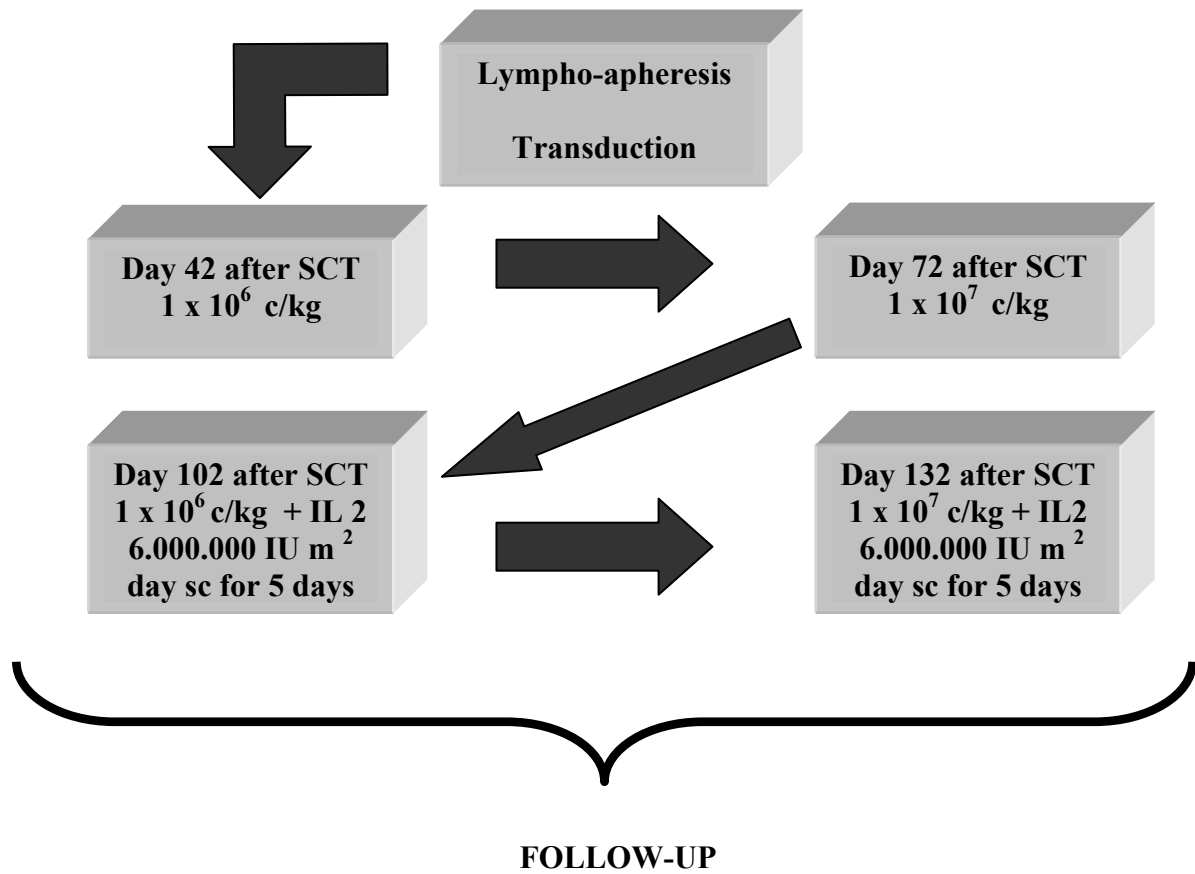
Study design page 17***Study plan******First infusion*****Previous version**

Administration of genetically modified donor lymphocytes at a dose of 1×10^6 cells/kg, starting at day+42 after SCT from haploidentical donor is planned in the absence of spontaneous immune reconstitution (documented by two consecutive findings of circulating CD3+ cells $> 100/\mu\text{l}$) and/or development of GvHD. In general the infusion (s) must be performed within an interval of 7 days from the planned day (except for patients under therapy with ganciclovir due to cytomegalovirus; in these cases the lymphocytes infusion must be performed 24 hours after the discontinuation of ganciclovir).

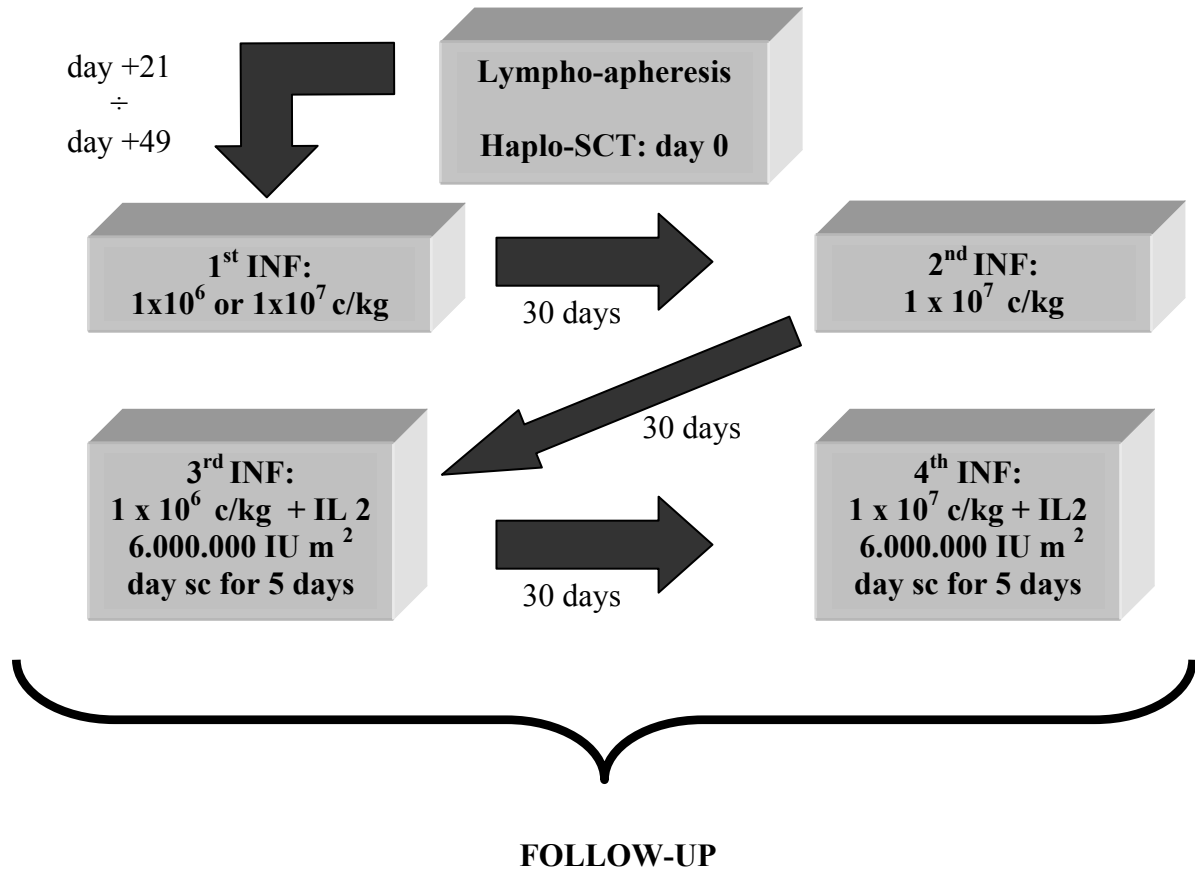
Next Version

Administration of genetically modified lymphocytes at a dose of 1×10^6 or 1×10^7 cells/kg, is planned in the absence of spontaneous immune reconstitution (documented by two consecutive findings of circulating CD3+ cells $> 100/\mu\text{l}$) and/or development of GvHD. In general, the infusion (s) must be performed within the interval between day+21 and day+49 after SCT.

Follow-up page 18
Previous Version



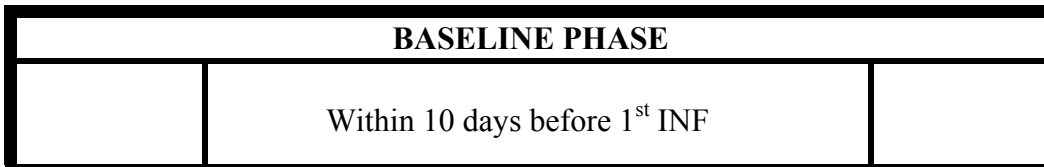
Next version



Procedures and treatment plan page 22
Screening phase and baseline phase
Previous version



Next version



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| MolMed S.p.A. | CLINICAL STUDY PROTOCOL | Internal Code: IPR/01.G |
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Appendix H: Flow chart protocol TK007

| | Screening (-30 day before SCT) | Baseline (+30 +40 day after SCT) | Follow up 1 st Month (day 14-21-28) | | | | Follow up 2 nd ,3 rd 4 th 5 th Month (day 14) | Follow up 6 th Month (day 28) | Post Follow up phase |
|--|--|--|--|---|---|---|---|--|-------------------------|
| Infusion of HSV-tk cells | | | | | | | | | |
| DLI to treat disease relapse | If clinically indicated and according to chapter 6.1 | | | | | | | | |
| Informed Consent | X | | | | | | | | |
| Physical examination | X | X | X | X | X | X | X | X | |
| Chest X ray | X | X | | | | | | | |
| Haematology | X | X | X | X | X | X | X | X | |
| Liver Function | X | X | X | X | X | X | X | X | |
| Renal Function | X | X | X | X | X | X | X | X | |
| Electrolytes | X | X | X | X | X | X | X | X | |
| Glycemia | X | X | X | X | X | X | X | X | |
| Total proteins | X | X | X | X | X | X | X | X | |
| Immunoglobulins | X | X | X | X | X | X | X | X | |
| IgG,IgM anti CMV | X | | | | | | X | X | |
| CMV monitoring | | X | X | X | X | X | X | X | |
| Serology HCV,HBV,EBV | X | | Monthly, at the end of study and when clinically indicated | | | | | | |
| Evaluation of heam. disease | X | X | Monthly, at the end of study and when clinically indicated | | | | | | |
| Spirometry | X | | Monthly, at the end of study and when clinically indicated | | | | | | |
| Citofluorimetry/ LNGFR (see chapter 9) | | X | | X | X | X | X | X | X |
| PCR HSV tk | | X | | X | X | X | X | X | X |
| Adverse Events | Throughout the trial (see chapter 11.1) | | | | | | | | |
| RCR | Pre-post treatment, at 3-6-12 months and yearly | | | | | | | | |

**Appendix I: Sponsor's responsibility
(requested by the Greek National Ethics Committee)**

1. MolMed S.p.A bears the responsibility for any direct or indirect damage that might be caused to the participant by the vector's administration or from any clinical intervention or procedure within the frames of his/her participation in the study, which would not have been carried out, if the participant had not taken part in the study. The company will not be responsible for any harmness associated with the transplantation procedure (transplant-related morbidity/mortality) in which the participant would have been anyway subjected according to medical judgment for his/her hematological malignancy and for which he/she would have signed a separate consent form.
2. MolMed S.p.A is also released from the above responsibility for damage, if the damage is due exclusively to any fault or misdemeanor of the participant or to not compliance to the given instructions.
3. For any claim of the participant in the above clinical trial against any competent or responsible person the competence lies with the Greek Courts.
4. The participant in the study surrenders from now on his/her claims against the above-mentioned responsible persons, to his/her insurance organization, should this insurance organization be financially burdened from the participation of the insured in the aforesaid study (indicatively and not exclusively, in case the insurance organization is charged with tests for the clinical study that wouldn't have been effectuated otherwise, with the value of the drug to be administered, the medical treatment or hospitalization because of complications due to the clinical trial, etc.).

Appendix L: Economic aspects
(requested by the Greek National Ethics Committee)

MolMed shall bear all the costs relating to the tests and visits foreseen by the clinical protocol. The costs are inclusive of all additional expenses for the treatments made on the patients by reason on their inclusion in the TK007 protocol, including laboratory procedures.

MolMed shall supply the investigational product, HSV-tk transduced donor lymphocytes, free of charge

Identification of the sponsor responsible for the purpose of this clinical trial:

MolMed S.p.A

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