A COMBINATION THERAPY FOR A STABLE AND LONG TERM ENGRAFTMENT

RELATED APPLICATION/S

U.S. Provisional Patent Application No. 61/578,917 filed on December 22, 2011, which is hereby incorporated by reference as if fully set forth herein.

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to a combination therapy for attaining a stable and long term cell or tissue transplantation.

The use of full-haplotype mismatched haploidentical donors as an alternative source for hematopoietic stem cell transplantation (HSCT) is highly attractive since virtually all patients have a readily available haploidentical family member that can serve as an HSCT donor. Early attempts to avoid fatal graft versus host disease (GVHD) risk and to apply haploidentical rigorously T cell depleted bone marrow transplantation (TDBMT) in leukemia patients revealed that the absence of donor T cells within the graft leads to a high rate of graft rejection, mediated by residual radiotherapy and chemotherapy resistant host-derived T cells (HTC). To overcome this obstacle, a 'mega dose' of TDBM cells was contemplated which can overcome this HTC mediated immune barrier and be engrafted successfully even when using fully mismatched murine strain combinations [Bachar-Lustig E et al., *Nat Med.* (1995) 1:1268-1273]. Subsequently, it was demonstrated that in humans, as in rodents, $CD34^+$ hematopoietic stem cell dose escalation may be used to overcome genetic barriers, enabling satisfactory survival rates following purified haploidentical HSCT [Reisner Y and Martelli MF. *Immunol Today.* (1995) 16:437-440 and U.S. Patent No. 5,806,529].

While the use of a purified 'mega dose' of $CD34^+$ HSCT has enabled haploidentical transplantation in leukemia patients, one major drawback, common to all T cell depleted transplants, is the slow recovery rate of the recipient's immune system. This is attributed to extensive immune ablating conditioning protocols prior to transplantation, the low numbers of donor T cells infused within the graft and to the decreased thymic function of adult recipients. Thus, in adult recipients of a haploidentical $CD34^+$ stem cell graft, a significant rate of transplant related mortality

(TRM) is caused by opportunistic infections.

Several approaches are being developed to address this challenge. This includes novel modalities to improve thymic function, post-transplant adoptive transfer of anti-viral specific T cells, transfer of partially polyclonal host-non-reactive allo-depleted T cells or transfer of fully polyclonal T cells transfected with inducible suicide genes. An alternative and additional approach to preserve host immunity is the use of reduced intensity conditioning (RIC). This non-myeloablative approach spares a substantial level of host immune cells and thus may reduce TRM by both improving post-transplant immune reconstitution and reducing the toxicity associated with the conditioning agents. Haploidentical transplantation under RIC is even more intricate due to the substantial immunological barrier presented by the surviving host T cells. Recent attempts to overcome this barrier, largely made use of non-T cell depleted grafts, which enable a high rate of engraftment, but in the expanse of increased rates of GVHD. Another approach for applying haploidentical transplantation under RIC uses CD3/CD19 depleted grafts, which not only contain $CD34^+$ stem cells but also CD34 negative progenitors, NK, graft facilitating cells and dendritic cells, however, this too is at the expanse of increased rates of GVHD and TRM.

In the 1970's George Santos demonstrated in rodents that a short course of high-dose cyclophosphamide (CY) soon after bone marrow transplant (BMT) targeted activated donor or host alloreactive T cells [Owens AH Jr and GW. S. *Transplantation.* (1971) 11:378-382]. Cyclophosphamide was observed to be non-toxic to hematopoietic stem cells because of their high expression of the detoxifying enzyme aldehyde dehydrogenase, and Slavin et al. further demonstrated that administration of high dose cyclophosphamide can reduce GVHD and graft rejection in mice, without adverse effects on stem cell engraftment [Brodsky RA and RJ. J. *Lancet.* (2005) 365:1647-1656]. Clinical trials by the John Hopkins and Fred Hutchinson Cancer Research Center groups, evaluated a non-myeloablative protocol of cyclophosphamide, fludarabine and 2Gy TBI, and post-transplant GVHD prophylaxis with cyclophosphamide (50 mg/kg days +3 and +4), MMF (days +5 to +35) and tacrolimus (days +5 to +180) [Luznik L et al., *Biology of blood and marrow transplantation: journal of the American Society for Blood and Marrow Transplantation.* (2008) 14:641]. According evident from their teachings, this protocol resulted in a high relapse

rate, which was probably due to poor disease debulking by the non-myeloablative conditioning and to lack of GVHD related graft versus leukemia (GVL) effect [Munchel A et al., *Pediatric Reports* (2011) 3:43-47].

Additional approaches for achieving stable engraftment of allogeneic hematopoietic stem cells have been attempted, some are described in U.S. Patent Application No. 20110110909, U.S. Patent Application No. 20050118142, U.S. Patent Application No. 20070098693, U.S. Patent No. 5,876,692, U.S. Patent No. 5,514,364, U.S. Patent No. 6,217,867, U.S. Patent No. 5,635,156, U.S. Patent Application No. 20060140912, U.S. Patent Application No. 20040005300, U.S. Patent Application No. 20070141027, U.S. Patent Application No. 20030017152, U.S. Patent Application No. 20030165475 and U.S. Patent Application No. 20010009663.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a method of treating a subject in need of a non-syngeneic cell or tissue graft, the method comprising: (a) transplanting into a subject a dose of T cell depleted immature hematopoietic cells, wherein the T cell depleted immature hematopoietic cells comprise less than 5 x 10^5 $CD3^+$ T cells per kilogram body weight of the subject, and wherein the dose comprises at least about 5 x 10^6 CD34+ cells per kilogram body weight of the subject; and subsequently (b) administering to the subject a therapeutically effective amount of cyclophosphamide, wherein the therapeutically effective amount comprises 25-200 mg per kilogram body weight, thereby treating the subject.

According to an aspect of some embodiments of the present invention there is provided a method of treating a subject in need of an immature hematopoietic cell transplantation, the method comprising: (a) transplanting into a conditioned subject a dose of T cell depleted immature hematopoietic cells, wherein the T cell depleted immature hematopoietic cells comprise less than 5 x 10^5 CD3+ T cells per kilogram body weight of the subject, and wherein the dose comprises at least about 5 x 10^6 CD34+ cells per kilogram body weight of the subject; and subsequently (b) administering to the subject a therapeutically effective amount of cyclophosphamide, wherein the therapeutically effective amount comprises 25-200 mg per kilogram body weight, thereby treating the subject.

According to an aspect of some embodiments of the present invention there is provided a method of treating a subject in need of an immature hematopoietic cell transplantation, the method comprising: (a) conditioning a subject under a reduced intensity conditioning protocol, wherein the reduced intensity conditioning comprises a total body irradiation (TBI) and a chemotherapeutic agent; (b) transplanting into the subject a dose of T cell depleted immature hematopoietic cells, wherein the T cell depleted immature hematopoietic cells comprise less than 5 x 10^5 CD3+ T cells per kilogram body weight of the subject, and wherein the dose comprises at least about 5 x 10^6 CD34+ cells per kilogram body weight of the subject; and subsequently (c) administering to the subject a therapeutically effective amount of cyclophosphamide, wherein the therapeutically effective amount comprises 25-200 mg per kilogram body weight, thereby treating the subject.

According to an aspect of some embodiments of the present invention there is provided a method of inducing donor specific tolerance in a subject in need of a non-syngeneic cell or tissue graft, the method comprising: (a) transplanting into a subject a dose of T cell depleted immature hematopoietic cells obtained from a non-syngeneic donor, wherein the T cell depleted immature hematopoietic cells comprise less than 5 x 10^5 $CD3^+$ T cells per kilogram body weight of the subject, and wherein the dose comprises at least about 5 x 10^6 CD34+ cells per kilogram body weight of the subject; and subsequently (b) administering to the subject a therapeutically effective amount of cyclophosphamide, wherein the therapeutically effective amount comprises 25-200 mg per kilogram body weight, thereby inducing donor specific tolerance in the subject.

According to some embodiments of the invention, the method further comprises conditioning the subject under reduced intensity conditioning prior to step (a).

According to some embodiments of the invention, the method further comprises conditioning the subject with *in-vivo* T cell debulking prior to step (a).

According to some embodiments of the invention, the dose of the T cell depleted immature hematopoietic cells comprises 5 - 40 x 10^6 CD34+ cells per kilogram body weight of the subject.

According to some embodiments of the invention, the dose of the T cell depleted immature hematopoietic cells comprises at least about 10 x 10^6 CD34+ cells per kilogram body weight of the subject.

According to some embodiments of the invention, the T cell depleted immature hematopoietic cells are selected from the group consisting of T cell depleted bone marrow cells, T cell depleted G-CSF mobilized peripheral blood progenitor cells, T cell depleted cord blood, purified CD34+ cells attained by positive selection from bone marrow and/or from G-CSF mobilized peripheral blood progenitor cells, and ex-vivo expanded $CD34^+$ cells.

According to some embodiments of the invention, the T cell depleted immature hematopoietic cells comprise less than 1 x 10^6 $CD8^+$ $TCR\alpha/\beta^-$ cells per kilogram body weight of the subject.

According to some embodiments of the invention, the T cell depleted immature hematopoietic cells are obtained by T cell debulking.

According to some embodiments of the invention, the T cell debulking is effected by antibodies.

According to some embodiments of the invention, the antibodies are selected from the group consisting of an anti-CD8 antibody, an anti-CD4 antibody, an anti-CD3 antibody, an anti-CD2 antibody and an anti-TCRα/β antibody.

According to some embodiments of the invention, the antibodies comprise an anti-CD3 antibody.

According to some embodiments of the invention, the immature hematopoietic cells are treated by B cell debulking.

According to some embodiments of the invention, the B cell debulking is effected by an anti-CD19 antibody or by anti-CD20 antibody.

According to some embodiments of the invention, the T cell depleted immature hematopoietic cells are obtained from a non-syngeneic donor.

According to some embodiments of the invention, the non-syngeneic donor is allogeneic or xenogeneic with respect to the subject.

According to some embodiments of the invention, the allogeneic donor is selected from the group consisting of an HLA matched sibling, an HLA matched unrelated donor, an HLA haploidentical related donor and a donor displaying one or more disparate HLA determinants.

According to some embodiments of the invention, the subject is a human subject.

According to some embodiments of the invention, the *in-vivo* T cell debulking is effected by antibodies.

According to some embodiments of the invention, the antibodies comprise an anti-CD8 antibody, an anti-CD4 antibody, or both.

According to some embodiments of the invention, the antibodies comprise anti-thymocyte globulin (ATG) antibodies, anti-CD52 antibodies or anti-CD3 (OKT3) antibodies.

According to some embodiments of the invention, the reduced intensity conditioning comprises a non-myeloablative conditioning.

According to some embodiments of the invention, the non-myeloablative conditioning comprises at least one of a total body irradiation (TBI), a total lymphoid irradiation (TLI), a chemotherapeutic agent and/or an antibody immunotherapy.

According to some embodiments of the invention, the TBI comprises a single or fractionated irradiation dose within the range of 1-7.5 Gy.

According to some embodiments of the invention, the TBI comprises a single or fractionated irradiation dose within the range of 1-3.5 Gy.

According to some embodiments of the invention, the TBI comprises a single or fractionated irradiation dose within the range of 2 Gy.

According to some embodiments of the invention, the TBI is effected in a single dose 2 days prior to step (b).

According to some embodiments of the invention, the chemotherapeutic agent comprises at least one of Busulfan, Fludarabine, Melphalan and Thiotepa.

According to some embodiments of the invention, the antibody comprises at least one of an anti-CD52 antibody, anti-thymocyte globulin (ATG) antibody or anti-CD3 (OKT3) antibody.

According to some embodiments of the invention, the concentration of the cyclophosphamide is about 100 - 200 mg per kg body weight.

According to some embodiments of the invention, the concentration of the cyclophosphamide is about 100 mg per kg body weight.

According to some embodiments of the invention, the cyclophosphamide is administered in a single dose.

According to some embodiments of the invention, the cyclophosphamide is administered in two doses.

According to some embodiments of the invention, each of the two doses comprises a concentration of about 50 mg per kg body weight.

According to some embodiments of the invention, each of the two doses is administered on days 3 and 4 following step (a).

According to some embodiments of the invention, the subject has a malignant disease.

According to some embodiments of the invention, the malignant disease is a hematopoietic cancer.

According to some embodiments of the invention, the hematopoietic cancer comprises a leukemia or lymphoma.

According to some embodiments of the invention, the hematopoietic cancer is selected from the group consisting of acute lymphoblastic leukemia (ALL), acute myelocytic leukemia (AML), acute nonlymphoblastic leukemia (ANLL), Chronic lymphocytic leukemia (CLL), chronic myelocytic leukemia (CML), Hodgkin's Lymphoma, non- Hodgkin's Lymphoma, Extranodal natural killer/T-cell lymphoma, Cutaneous T-cell lymphoma, Enteropathy type T-cell lymphoma, Angioimmunoblastic T-cell lymphoma, Anaplastic large T/null-cell lymphoma, Subcutaneous panniculitis-like T-cell lymphoma, Unspecified T-cell lymphoma, Diffuse large B-cell lymphoma, B-cell chronic lymphocytic leukemia (B-CLL)/chronic lymphoid leukemia (CLL), Chronic lymphocytic leukemia/small lymphocytic lymphoma, Extranodal marginal zone B-cell lymphomas - mucosa-associated lymphoid tissue lymphomas, Follicular lymphoma, Mantle cell lymphoma, Nodal marginal zone B-cell lymphoma, Burkitt lymphoma, Hairy cell leukemia, Primary central nervous system lymphoma, Splenic marginal zone B-cell lymphoma, Lymphoplasmocytic lymphoma, Primary mediastinal B-cell lymphoma and multiple myeloma.

According to some embodiments of the invention, the subject has a non-malignant disease.

According to some embodiments of the invention, the non-malignant disease is a genetic disease or disorder, an autoimmune disease or a metabolic disorder.

According to some embodiments of the invention, the non-malignant disease is selected from the group consisting of sickle cells disease, a congenital neutropenia, a thrombocytopenia, an aplastic anemia, a myelodysplastic syndrome, a monosomy 7, an osteopetrosis, a Gaucher's disease, a Hurler's disease, a metachromatic leukodystrophy, an adrenal leukodystrophy, a thalassemia, a congenital or genetically-determined hematopoietic abnormality, lupus, autoimmune hepatitis, celiac disease, type I diabetes mellitus, Grave's disease, Guillain-Barr syndrome, Myasthenia gravis, Rheumatoid arthritis, scleroderma and psoriasis.

According to some embodiments of the invention, the cell or tissue graft comprises immature hematopoietic cells.

According to some embodiments of the invention, the cell or tissue graft is selected from the group consisting of a liver, a pancreas, a spleen, a kidney, a heart, a lung, a skin, an intestine and a lymphoid/hematopoietic tissue or organ.

According to some embodiments of the invention, the cell or tissue graft is transplanted into the subject prior to, concomitantly with or following the transplanting the dose of T cell depleted immature hematopoietic cells into the subject.

According to some embodiments of the invention, the cell or tissue graft comprises a co-transplantation of several organs.

According to some embodiments of the invention, the cell or tissue graft and the T cell depleted immature hematopoietic cells are obtained from the same donor.

According to some embodiments of the invention, the conditioned subject has been conditioned under reduced intensity conditioning.

According to some embodiments of the invention, the reduced intensity conditioning is effected 1-10 days prior to the transplanting.

According to some embodiments of the invention, the conditioned subject has been conditioned with *in-vivo* T cell debulking.

According to some embodiments of the invention, the *in-vivo* T cell debulking is effected 4-7 days prior to the transplanting.

According to some embodiments of the invention, the chemotherapeutic agent comprises Fludarabine.

According to some embodiments of the invention, the Fludarabine is effected at a dose of 30 $mg/m^2/day$.

According to some embodiments of the invention, the Fludarabine is administered daily on days 3 to 7 prior to step (b).

According to some embodiments of the invention, the T cell depleted immature hematopoietic cells comprise T cell depleted G-CSF mobilized peripheral blood progenitor cells.

It will be appreciated that the present teachings can be used with other tolerance inducing protocols such as described in PCT publication Nos. WO 2001/49243, WO 2007/023491 and WO 2010/049935, which are herein incorporated by reference in their entirety.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A-B are graphs illustrating durable engraftment of mismatched donor bone marrow (BM) following transplantation of 'mega dose' rigorously T cell depleted BM and posttranplantation cyclophosphamide. Mice were conditioned with T cell debulking (TCD), using anti-CD4 and anti-CD8 antibodies, on day -6, and by exposure to 2.0 Gy total body irradiation (TBI) on day -1. High dose Cyclophosphamide (CY, 100 mg/kg) was administered on days +3 and +4 post transplant. Donor type chimerism was evaluated 35 days (Figure 1A) and 95 days (Figure 1B) post transplant.

FIGs. 2A-C are dot plot graphs illustrating a typical FACS chimerism analysis. Figure 2C depicts that mixed chimerism was achieved in recipients that were transplanted with 'mega dose' (25 x 10^6) rigorously T cell depleted BM and were treated with high dose CY. In contrast, recipient mice that received only the conditioning protocol (Figure 2A) or which were inoculated with only 5 x 10^6 BM cells and CY did not exhibit donor type chimerism (Figure 2B).

FIG. 3 is a graph illustrating durable mixed chimerism 180 and 225 days post transplant in recipient mice that were transplanted with 'mega dose' (25 x 10^6) T cell depleted BM and were treated with high dose CY. Of note, mice which were inoculated with 5 x 10^6 T cell depleted BM and CY did not exhibit mixed chimerism.

FIGs. 4A-B illustrate the transplantation of donor type or 3rd party skin grafts in chimeric mice. Figure 4A is a graft illustrating acceptance (marked by "+") or rejection (marked by "–") of donor type (Balb/c) or 3rd party (C57BL/6) skin grafts in recipients of regular dose (5 x 10^6) or 'mega dose' (25 x 10^6) T depleted BM, treated with high dose CY on days +3 and +4 post transplant. Figure 4B is a photograph of donor type (Balb/c) skin graft (white fur) or 3rd party (C57BL/6) skin graft (black fur) in recipients of 'mega dose' (25 x 10^6) T depleted BM, treated with high dose CY on days +3 and +4 post transplant.

FIG. 5 is a graph illustrating the effect of different doses of irradiation on donor type chimerism in recipient mice of 'mega dose' (25 x 10^6) T depleted BM and treated with high dose CY post transplant.

FIG. 6 is a graph illustrating the effect of increased doses of Cyclophosphamide (CY) on donor type chimerism in recipients of 'mega dose' (25 x 10^6) T depleted BM and 2 Gy TBI.

FIG. 7 is a graph illustrating engraftment of mismatched donor BM achived by combining 'mega dose' $CD8^+$ T cell depleted BM and post trasplant CY. Of note, the depletion of residual $CD8^+$ T cells from the BM preparation did not have any adverse impact on the level of chimerism achieved when combing 'mega dose' T cell depleted BM cells with post transplant CY.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to a combination therapy for attaining a stable and long term cell or tissue transplantation.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Application of allogeneic hematopoietic stem cell transplantation (HSCT) has been limited by the lack of available HLA-matched donors within the family or in the international registries of unrelated volunteer donors. Conversely, virtually all patients in need for a transplant have a full-haplotype mismatched family donor.

The major obstacles to bone marrow transplantation from full-haplotype mismatched related donors were graft versus host disease (GVHD) and graft rejection. The use of very large numbers of hematopoietic stem cells with minimal residual T cell contamination and an aggressive immunosuppressive and myeloablative regimen has resulted in high rates of engraftment with little severe GVHD. However, immune reconstitution has been delayed and incomplete after this approach and a significant rate of transplant related mortality (TRM) is caused by opportunistic infections.

While reducing the present invention to practice, the present inventors have uncovered that a successful engraftment of mismatched bone marrow can be achieved by transplantation of rigorously T cell depleted 'mega dose' bone marrow and subsequently administering to the subject a high-dose cyclophospamide early after transplantation. The present inventors have shown that such a regimen requires only a short immunomyeloablative conditioning regimen. The present inventors have further shown that such a transplantation procedure leads to a long and stable chimerism and that tolerance has been achieved.

As is shown hereinbelow and in the Examples section which follows, the present inventors have uncovered through laborious experimentation that the combination of

'mega dose' T cell depleted bone marrow transplantation (TDBMT) and post transplant high dose cyclophosphamide (CY) allows for a durable engraftment of mismatched donor bone marrow (see Figures 1A-B and 2A-C). Durable mixed chimerism was exhibited for prolonged periods of time after transplantation (180 and 225 days post transplant in mice, see Figure 3). Importantly, the combination of 'mega dose' TDBMT and high dose CY following transplantation allowed hematopoietic stem cell engraftment under reduced intensity conditioning (see Figure 5) and resulted in tolerance induction, as indicated by acceptance of donor skin grafts (see Figure 4B).

Thus, according to one aspect of the present invention there is provided a method of treating a subject in need of a non-syngeneic cell or tissue graft, the method comprising: (a) transplanting into a subject a dose of T cell depleted immature hematopoietic cells, wherein the T cell depleted immature hematopoietic cells comprise less than 5 x 10^5 $CD3^+$ T cells per kilogram body weight of the subject, and wherein the dose comprises at least about 5 x 10^6 CD34+ cells per kilogram body weight of the subject; and subsequently (b) administering to the subject a therapeutically effective amount of cyclophosphamide, wherein the therapeutically effective amount comprises 25-200 mg per kilogram body weight, thereby treating the subject.

As used herein, the term "treating" includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

As used herein, the term "subject" or "subject in need thereof" refers to a mammal, preferably a human being, male or female at any age that is in need of a cell or tissue transplantation. Typically the subject is in need of cell or tissue transplantation (also referred to herein as recipient) due to a disorder or a pathological or undesired condition, state, or syndrome, or a physical, morphological or physiological abnormality which is amenable to treatment via cell or tissue transplantation.

According to an embodiment the subject is in need of tissue regeneration (solid or soft tissue) such as due to aging, trauma, wound or any pathological condition which results in loss of organ functionality.

According to one embodiment the subject has a malignant disease.

According to one embodiment the malignant disease is a hematopoietic cancer.

Exemplary hematopoietic cancers include, but are not limited to, acute lymphoblastic leukemia (ALL), T-cell acute lymphocytic leukemia (T-ALL), acute myelocytic leukemia (AML), acute nonlymphoblastic leukemia (ANLL), chronic lymphocytic leukemia (CLL), chronic myelocytic leukemia (CML), T-cell prolymphocytic leukemia, B-cell prolymphocytic leukemia, Juvenile myelomonocytic leukemia, Hodgkin's Lymphoma, non- Hodgkin's Lymphoma, Extranodal natural killer/T-cell lymphoma, Cutaneous T-cell lymphoma, Enteropathy type T-cell lymphoma, Angioimmunoblastic T-cell lymphoma, Anaplastic large T/null-cell lymphoma, Subcutaneous panniculitis-like T-cell lymphoma, Unspecified T-cell lymphoma, Diffuse large B-cell lymphoma (DLBCL), B-cell chronic lymphocytic leukemia (B-CLL)/chronic lymphoid leukemia (CLL), Chronic lymphocytic leukemia/small lymphocytic lymphoma, Extranodal marginal zone B-cell lymphomas - mucosa-associated lymphoid tissue lymphomas, Follicular lymphoma, Mantle cell lymphoma, Nodal marginal zone B-cell lymphoma, Burkitt lymphoma, Hairy cell leukemia, Primary central nervous system lymphoma, Splenic marginal zone B-cell lymphoma, Lymphoplasmocytic lymphoma, Primary mediastinal B-cell lymphoma, precursor T-cell leukemia/lymphoma, MALT lymphoma, Mycosis fungoides and multiple myeloma.

According to one embodiment the hematopoietic cancer comprises a leukemia or a lymphoma.

According to one embodiment the subject has a non-malignant disease.

According to one embodiment the non-malignant disease is a genetic disease or disorder, an autoimmune disease or a metabolic disorder.

Exemplary non-malignant diseases include, but are not limited to, severe combined immunodeficiency syndromes (SCID), sickle cell disease (sickle cell anemia), congenital neutropenia, thrombocytopenia, aplastic anemia (e.g. severe aplastic anemia), myelodysplastic syndrome, monosomy 7, osteopetrosis, Gaucher's disease, Hurler's disease, metachromatic leukodystrophy, adrenal leukodystrophy, thalassemia, congenital or genetically-determined hematopoietic abnormality, adenosine deaminase (ADA), lupus, autoimmune hepatitis, celiac disease, type I diabetes mellitus, Grave's disease, Guillain-Barr syndrome, Myasthenia gravis, Rheumatoid arthritis, scleroderma and psoriasis.

According to one embodiment the subject of the present invention may suffer from any of a cardiovascular disease, a rheumatoid disease, a glandular disease, a gastrointestinal disease, a cutaneous disease, a hepatic disease, a neurological disease, a muscular disease, a nephric disease, a connective tissue disease, a systemic disease and/or a disease related to reproduction, treatable by cell or tissue transplantation.

As used herein, the phrase "cell or tissue graft" refers to a bodily cell (e.g. a single cell or a group of cells) or tissue (e.g. solid tissues or soft tissues, which may be transplanted in full or in part). Exemplary tissues which may be transplanted according to the present teachings include, but are not limited to, liver, pancreas, spleen, kidney, heart, lung, skin, intestine and lymphoid/hematopoietic tissues (e.g. lymph node, Peyer's patches, thymus or bone marrow). Exemplary cells which may be transplanted according to the present teachings include, but are not limited to, immature hematopoietic cells including stem cells. The present invention also contemplates transplantation of whole organs, such as for example, kidney, heart, lung, liver, pancreas or spleen.

According to one embodiment, the cell or tissue graft comprises immature hematopoietic cells.

According to one embodiment, the method is effected using a cell or tissue, which is non-syngeneic with the subject.

Depending on the application, the method may be effected using a cell or tissue graft which is allogeneic or xenogeneic with the subject.

As used herein, the term "allogeneic" refers to a cell or tissue which is derived from a donor who is of the same species as the subject, but which is substantially non-clonal with the subject. Typically, outbred, non-zygotic twin mammals of the same species are allogeneic with each other. It will be appreciated that an allogeneic donor may be HLA identical or HLA non-identical (i.e. displaying one or more disparate HLA determinants) with respect to the subject.

According to one embodiment, the allogeneic donor is an HLA matched sibling, an HLA matched unrelated donor, an HLA haploidentical related donor or a donor displaying one or more disparate HLA determinants.

As used herein, the term "xenogeneic" refers to a cell or tissue which substantially expresses antigens of a different species relative to the species of a

substantial proportion of the lymphocytes of the subject. Typically, outbred mammals of different species are xenogeneic with each other.

The present invention envisages that xenogeneic cells or tissues are derived from a variety of species such as, but not limited to, bovines (e.g., cow), equines (e.g., horse), porcines (e.g. pig), ovids (e.g., goat, sheep), felines (e.g., Felis domestica), canines (e.g., Canis domestica), rodents (e.g., mouse, rat, rabbit, guinea pig, gerbil, hamster) or primates (e.g., chimpanzee, rhesus monkey, macaque monkey, marmoset).

Cells or tissues of xenogeneic origin (e.g. porcine origin) are preferably obtained from a source which is known to be free of zoonoses, such as porcine endogenous retroviruses. Similarly, human-derived cells or tissues are preferably obtained from substantially pathogen-free sources.

According to an embodiment of the present invention, both the subject and the donor are humans.

Depending on the application and available sources, the cell or tissue graft of the present invention may be obtained from a prenatal organism, postnatal organism, an adult or a cadaver donor. Moreover, depending on the application needed, the cell or tissue graft may be naïve or genetically modified. Determination of the type of cell or tissue graft to be used is well within the ability of one of ordinary skill in the art. Furthermore, any method known in the art may be employed to obtain a cell or tissue graft (e.g. for transplantation).

As mentioned, a dose of T cell depleted hematopoietic cell or tissue comprising immature hematopoietic cells (including e.g. $CD34^+$), are transplanted into a subject.

According to one embodiment, the T cell depleted immature hematopoietic cells are non-syngeneic (e.g. allogeneic or xenogeneic) with the subject.

According to one embodiment, the T cell depleted immature hematopoietic cells and the cell or tissue graft are syngeneic (e.g. obtained from the same donor).

As used herein the phrase "immature hematopoietic cells" refers to a hematopoietic tissue or cell preparation comprising precursor hematopoietic cells. Such tissue/cell preparation includes or is derived from a biological sample, for example, bone marrow, mobilized peripheral blood (e.g. mobilization of CD34 cells to enhance their concentration), cord blood (e.g. umbilical cord), fetal liver, yolk sac and/or placenta. Additionally, purified CD34+ cells or other hematopoietic stem cells such as CD131+

cells can be used in accordance with the present teachings, either with or without *ex-vivo* expansion.

According to one embodiment, the immature hematopoietic cells comprise T cell depleted immature hematopoietic cells.

As used herein the phrase "T cell depleted immature hematopoietic cells" refers to a population of hematopoietic cells which are depleted of T lymphocytes. The T cell depleted immature hematopoietic cells, may include e.g. CD34+, CD33+ and/or CD56+ cells. The T cell depleted immature hematopoietic cells may be depleted of CD3+ cells, CD2+ cells, CD8+ cells, CD4+ cells, α/β T cells and/or γ/δ T cells.

According to one embodiment, the immature hematopoietic cells comprise T cell depleted G-CSF mobilized blood cells enriched for $CD34^+$ immature hematopoietic cells.

According to one embodiment, the immature hematopoietic cells are depleted of CD3+ T cells.

According to an embodiment, the T cell depleted immature hematopoietic cells comprise less than 50×10^5 $CD3^+$ T cells, 40×10^5 $CD3^+$ T cells, 30×10^5 $CD3^+$ T cells, 20×10^5 $CD3^+$ T cells, 15×10^5 $CD3^+$ T cells, 10×10^5 $CD3^+$ T cells, 9×10^5 $CD3^+$ T cells, 8×10^5 $CD3^+$ T cells, 7×10^5 $CD3^+$ T cells, 6×10^5 $CD3^+$ T cells, 5×10^5 $CD3^+$ T cells, 4×10^5 $CD3^+$ T cells, 3×10^5 $CD3^+$ T cells, 2×10^5 $CD3^+$ T cells or 1×10^5 $CD3^+$ T cells per kilogram body weight of the subject.

According to a specific embodiment, the T cell depleted immature hematopoietic cells comprise less than 5×10^5 $CD3^+$ T cells per kilogram body weight of the subject.

According to a specific embodiment the T cell depleted immature hematopoietic cells comprise less than 20×10^5 $CD3^+$ T cells but more than 10 $CD3^+$ T cells.

According to an embodiment, the T cell depleted immature hematopoietic cells comprise at least 1×10^3 - 1×10^5 $CD3^+$ T cells.

According to one embodiment, the immature hematopoietic cells are depleted of CD8+ cells.

According to an embodiment, the T cell depleted immature hematopoietic cells comprise less than $1 \times 10^4 - 4 \times 10^5$ $CD8^+$ cells per kilogram body weight of the subject.

According to an embodiment, the T cell depleted immature hematopoietic cells comprise less than 50×10^5 $CD8^+$ cells, 25×10^5 $CD8^+$ cells, 15×10^5 $CD8^+$ cells, $10 \times$

10^5 $CD8^+$ cells, 9 x 10^5 $CD8^+$ cells, 8 x 10^5 $CD8^+$ cells, 7 x 10^5 $CD8^+$ cells, 6 x 10^5 $CD8^+$ cells, 5 x 10^5 $CD8^+$ cells, 4 x 10^5 $CD8^+$ cells, 3 x 10^5 $CD8^+$ cells, 2 x 10^5 $CD8^+$ cells, 1 x 10^5 $CD8^+$ cells, 9 x 10^4 $CD8^+$ cells, 8 x 10^4 $CD8^+$ cells, 7 x 10^4 $CD8^+$ cells, 6 x 10^4 $CD8^+$ cells, 5 x 10^4 $CD8^+$ cells, 4 x 10^4 $CD8^+$ cells, 3 x 10^4 $CD8^+$ cells, 2 x 10^4 $CD8^+$ cells or 1 x 10^4 $CD8^+$ cells per kilogram body weight of the subject.

According to a specific embodiment, the T cell depleted immature hematopoietic cells comprise less than 4 x 10^5 $CD8^+$ cells per kilogram body weight of the subject.

According to a specific embodiment the T cell depleted immature hematopoietic cells comprise less than 4 x 10^5 $CD8^+$ cells but more than 10 $CD8^+$ cells.

According to an embodiment, the T cell depleted immature hematopoietic cells comprise less than 1 x 10^6 $CD8^+$ TCRα/β- cells per kilogram body weight of the subject.

According to an embodiment, the T cell depleted immature hematopoietic cells comprise less than 1 x 10^6 $CD8^+$ $TCR\alpha/\beta^-$ cells, 0.5 x 10^6 $CD8^+$ $TCR\alpha/\beta^-$ cells, 1 x 10^5 $CD8^+$ $TCR\alpha/\beta^-$ cells, 0.5 x 10^5 $CD8^+$ $TCR\alpha/\beta^-$ cells, 1 x 10^4 $CD8^+$ $TCR\alpha/\beta^-$ cells, 0.5 x 10^4 $CD8^+$ $TCR\alpha/\beta^-$ cells, 1 x 10^3 $CD8^+$ $TCR\alpha/\beta^-$ cells or 0.5 x 10^3 $CD8^+$ $TCR\alpha/\beta^-$ cells per kilogram body weight of the subject.

According to a specific embodiment, the T cell depleted immature hematopoietic cells comprise less than 1 x 10^6 $CD8^+$ $TCR\alpha/\beta^-$ cells per kilogram body weight of the subject.

According to a specific embodiment the T cell depleted immature hematopoietic cells comprise less than 1 x 10^6 $CD8^+$ $TCR\alpha/\beta^-$ cells but more than 10 $CD8^+$ $TCR\alpha/\beta^-$ cells.

According to one embodiment, the immature hematopoietic cells are depleted of B cells.

According to an embodiment, the immature hematopoietic cells are depleted of B cells (CD19+ and/or CD20+ B cells).

According to an embodiment, the immature hematopoietic cells comprise less than 50 x 10^5 B cells, 40 x 10^5 B cells, 30 x 10^5 B cells, 20 x 10^5 B cells, 10 x 10^5 B cells, 9 x 10^5 B cells, 8 x 10^5 B cells, 7 x 10^5 B cells, 6 x 10^5 B cells, 5 x 10^5 B cells, 4 x 10^5 B cells, 3 x 10^5 B cells, 2 x 10^5 B cells or 1 x 10^5 B cells per kilogram body weight of the subject.

According to a specific embodiment, the immature hematopoietic cells comprise less than 4 x 10^5 B cells per kilogram body weight of the subject. According to a specific embodiment the immature hematopoietic cells comprise less than 50 x 10^5 B cells but more than 10 B cells.

Depletion of T cells, e.g. CD3+, CD2+, TCRα/β+, CD4+ and/or CD8+ cells, or B cells, e.g. CD19+ and/or CD20+ cells, may be carried out using any method known in the art, such as by eradication (e.g. killing) with specific antibodies or by affinity based purification e.g. such as by the use of magnetic cell separation techniques, FACS sorter and/or capture ELISA labeling.

Such methods are described herein and in THE HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, Volumes 1 to 4, (D.N. Weir, editor) and FLOW CYTOMETRY AND CELL SORTING (A. Radbruch, editor, Springer Verlag, 1992). For example, cells can be sorted by, for example, flow cytometry or FACS. Thus, fluorescence activated cell sorting (FACS) may be used and may have varying degrees of color channels, low angle and obtuse light scattering detecting channels, and impedance channels. Any ligand-dependent separation techniques known in the art may be used in conjunction with both positive and negative separation techniques that rely on the physical properties of the cells rather than antibody affinity, including but not limited to elutriation and density gradient centrifugation.

Other methods for cell sorting include, for example, panning and separation using affinity techniques, including those techniques using solid supports such as plates, beads and columns. Thus, biological samples may be separated by "panning" with an antibody attached to a solid matrix, e.g. to a plate.

Alternatively, cells may be sorted/separated by magnetic separation techniques, and some of these methods utilize magnetic beads. Different magnetic beads are available from a number of sources, including for example, Dynal (Norway), Advanced Magnetics (Cambridge, MA, U.S.A.), Immuncon (Philadelphia, U.S.A.), Immunotec (Marseille, France), Invitrogen, Stem cell Technologies (U.S.A) and Cellpro (U.S.A). Alternatively, antibodies can be biotinylated or conjugated with digoxigenin and used in conjunction with avidin or anti-digoxigenin coated affinity columns.

According to an embodiment, different depletion/separation methods can be combined, for example, magnetic cell sorting can be combined with FACS, to increase the separation quality or to allow sorting by multiple parameters.

According to one embodiment, the T cell depleted immature hematopoietic cells are obtained by T cell debulking (TCD).

T cell debulking may be effected using antibodies, including e.g. anti-CD8 antibodies, anti-CD4 antibodies, anti-CD3 antibodies, anti-CD2 antibodies, anti-TCRα/β antibodies and/or anti-TCRγ/δ antibodies.

According to one embodiment, depletion of B cells is effected by B cell debulking.

B cell debulking may be effected using antibodies, including e.g. anti-CD19 or anti-CD20 antibodies. Alternatively, debulking *in-vivo* of B cells can be attained by infusion of anti-CD20 antibodies.

Alternatively, positive selection of CD34+ or CD131+ stem cells may be carried out using e.g. magnetic cell separation techniques, FACS sorter and/or capture ELISA labeling as described in further detail above.

As mentioned, T cell or B cell debulking may be effected *in-vitro* or *in-vivo* (e.g. in a donor prior to acquiring immature hematopoietic cells therefrom).

According to one embodiment, the T cell depleted immature hematopoietic cells (e.g. comprising CD34+ cells) comprise T cell depleted bone marrow cells, T cell depleted mobilized peripheral blood progenitor cells (e.g. mobilized by G-CSF), T cell depleted cord blood/fetal liver/yolk sac and/or, purified $CD34^+$ cells (harvested from all the sources mentioned above e.g. from bone marrow and/or from G-CSF mobilized peripheral blood progenitor cells) and selected by positive selection (e.g. with magnetic beads using an anti-CD34 antibody). In addition purified $CD34^+$ cells expanded ex-vivo to increase cell numbers are also contemplated by the present methods.

According to an embodiment of the present invention, the subject is administered with a dose of T cell depleted immature hematopoietic cells comprising at least about, 4 x 10^6, 4.5 x 10^6, 5 x 10^6, 5.5 x 10^6, 6 x 10^6, 6.5 x 10^6, 7 x 10^6, 7.5 x 10^6, 8 x 10^6, 8.5 x 10^6, 9 x 10^6, 9.5 x 10^6, 10 x 10^6, 12.5 x 10^6, 15 x 10^6, 20 x 10^6, 25 x 10^6, 30 x 10^6, 35 x 10^6, 40 x 10^6, 45 x 10^6, 50 x 10^6, 60 x 10^6, 70 x 10^6, 80 x 10^6, 90 x 10^6 CD34+ cells per kilogram body weight.

According to a specific embodiment, the subject is administered a dose of T cell depleted immature hematopoietic cells comprising at least about 10 x 10^6 CD34+ cells per kilogram body weight.

According to a specific embodiment, the subject is administered a dose of T cell depleted immature hematopoietic cells comprising at least about 5 x 10^6 CD34+ cells per kilogram body weight.

According to one embodiment, the subject is administered a dose of T cell depleted immature hematopoietic cells comprising a range of about 4-30 x 10^6, 4-40 x 10^6, 4-50 x 10^6, 4-60 x 10^6, 4-70 x 10^6, 4-80 x 10^6, 4-90 x 10^6, 4-100 x 10^6, 5-10 x 10^6, 5-20 x 10^6, 5-30 x 10^6, 5-40 x 10^6, 5-50 x 10^6, 5-60 x 10^6, 5-70 x 10^6, 5-80 x 10^6, 5-90 x 10^6, 5-100 x 10^6, 10-20 x 10^6, 10-30 x 10^6, 10-40 x 10^6, 10-50 x 10^6, 10-60 x 10^6, 10-70 x 10^6, 10-80 x 10^6, 10-90 x 10^6, 10-100 x 10^6, 20-30 x 10^6, 20-40 x 10^6, 20-50 x 10^6, 20-60 x 10^6, 20-70 x 10^6, 20-80 x 10^6, 20-90 x 10^6, 20-100 x 10^6, 30-40 x 10^6, 30-50 x 10^6, 30-60 x 10^6, 30-70 x 10^6, 30-80 x 10^6, 30-90 x 10^6, 30-100 x 10^6, 40-50 x 10^6, 40-60 x 10^6, 40-70 x 10^6, 40-80 x 10^6, 40-90 x 10^6, 40-100 x 10^6, 50-60 x 10^6, 50-70 x 10^6, 50-80 x 10^6, 50-90 x 10^6, 50-100 x 10^6, 60-70 x 10^6, 60-80 x 10^6, 60-90 x 10^6, 60-100 x 10^6, 70-80 x 10^6, 70-90 x 10^6, 70-100 x 10^6, 80-90 x 10^6, 80-100 x 10^6 CD34+ cells per kilogram body weight of the subject.

According to a specific embodiment, the subject is administered a dose of T cell depleted immature hematopoietic cells comprising a range of about 5-40 x 10^6 CD34+ cells per kilogram body weight.

The T cell depleted immature hematopoietic cells of the present invention may be transplanted into a recipient using any method known in the art for cell transplantation, such as but not limited to, cell infusion (e.g. I.V.), via an intraperitoneal route or via an intrabone route.

As mentioned, the subject of the instant invention may further be transplanted with a cell or tissue graft (e.g. liver, pancreas, spleen, kidney, heart, lung, skin, intestine and/or lymphoid/hematopoietic tissues).

Transplanting the cell or tissue into the subject may be effected in numerous ways, depending on various parameters, such as, for example, the cell or tissue type; the type, stage or severity of the recipient's disease (e.g. organ failure); the physical or physiological parameters specific to the subject; and/or the desired therapeutic outcome.

Transplanting a cell or tissue graft of the present invention may be effected by transplanting the cell or tissue graft into any one of various anatomical locations, depending on the application. The cell or tissue graft may be transplanted into a homotopic anatomical location (a normal anatomical location for the transplant), or into an ectopic anatomical location (an abnormal anatomical location for the transplant). Depending on the application, the cell or tissue graft may be advantageously implanted under the renal capsule, or into the kidney, the testicular fat, the sub cutis, the omentum, the portal vein, the liver, the spleen, the heart cavity, the heart, the chest cavity, the lung, the skin, the pancreas and/or the intra abdominal space.

For example, a liver tissue according to the present teachings may be transplanted into the liver, the portal vein, the renal capsule, the sub-cutis, the omentum, the spleen, and the intra-abdominal space. Transplantation of a liver into various anatomical locations such as these is commonly practiced in the art to treat diseases amenable to treatment via hepatic transplantation (e.g. hepatic failure). Similarly, transplanting a pancreatic tissue according to the present invention may be advantageously effected by transplanting the tissue into the portal vein, the liver, the pancreas, the testicular fat, the sub-cutis, the omentum, an intestinal loop (the subserosa of a U loop of the small intestine) and/or the intra-abdominal space. Transplantation of pancreatic tissue may be used to treat diseases amenable to treatment via pancreatic transplantation (e.g. diabetes). Likewise, transplantation of tissues such as a kidney, a heart, a lung or skin tissue may be carried out into any anatomical location described above for the purpose of treating recipients suffering from, for example, renal failure, heart failure, lung failure or skin damage (e.g., burns).

Optionally, when transplanting a cell or tissue graft of the present invention into a subject having a defective organ, it may be advantageous to first at least partially remove the failed organ from the subject so as to enable optimal development of the transplant, and structural/functional integration thereof with the anatomy/physiology of the subject.

The method of the present invention also envisions co-transplantation of several organs (e.g. heart and lung, liver and spleen, pancreas and bone marrow e.g. hematopoietic stem cells, kidney and bone marrow e.g. hematopoietic stem cells, etc.) in case the subject may be beneficially affected by such a procedure.

According to one embodiment, the co-transplantation comprises transplantation of immature hematopoietic cells and a solid tissue/organ or a number of solid organs/tissues.

According to one embodiment, the immature hematopoietic cells and the solid organ are obtained from the same donor.

According to one embodiment, the cell or tissue graft (e.g. solid organ) is transplanted into the subject prior to, concomitantly with or following transplanting of the T cell depleted immature hematopoietic cells (e.g. comprising CD34+ cells) into the subject.

Following transplantation of the cell or tissue graft into the subject, it is advisable, according to standard medical practice, to monitor the growth functionality and immuno-compatability of the organ according to any one of various standard art techniques. For example, the functionality of a pancreatic tissue transplant may be monitored following transplantation by standard pancreas function tests (e.g. analysis of serum levels of insulin). Likewise, a liver tissue transplant may be monitored following transplantation by standard liver function tests (e.g. analysis of serum levels of albumin, total protein, ALT, AST, and bilirubin, and analysis of blood-clotting time). Structural development of the cell or tissue graft may be monitored via computerized tomography, or ultrasound imaging.

Regardless of the transplant type, in order to reduce, by at least about 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 % or 95 %, or preferably avoid graft rejection and/or graft versus host disease (GVHD), the present invention contemplates post transplant administration of cyclophosphamide.

According to one embodiment, the present invention further contemplates administration of cyclophosphamide prior to transplantation (e.g. on days 4, 3 or 2 prior to transplantation, i.e. T-4, -3 or -2) in addition to the administration following transplantation as described herein.

Of note, the date of transplantation (of the cell or tissue graft) is considered T=zero.

As used herein, the term "cyclophosphamide" refers to the nitrogen mustard alkylating agent which specifically adds an alkyl group (C_nH_{2n+1}) to DNA (also known as cytophosphane). In a specific embodiment, the cyclophosphamide refers to the

molecular formula $C_7H_{15}Cl_2N_2O_2P \cdot H_2O$ and the chemical name 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate. Cyclophosphamide is commercially available from e.g. Zydus (German Remedies), Roxane Laboratories Inc-Boehringer Ingelheim, Bristol-Myers Squibb Co - Mead Johnson and Co, and Pfizer - Pharmacia & Upjohn, under the brand names of Endoxan, Cytoxan, Neosar, Procytox and Revimmune.

A therapeutically effective amount of cyclophosphamide is typically administered to the subject following transplantation of the cell or tissue graft.

Without being bound to theory, a therapeutically effective amount is an amount of cyclophosphamide efficient for killing activated donor or host alloreactive T cells without being toxic to the subject.

For example, in case of cell or tissue graft, the therapeutic effective amount of cyclophosphamide comprises about 1-25 mg, 1-50 mg, 1-75 mg, 1-100 mg, 1-250 mg, 1-500 mg, 1-750 mg, 1-1000 mg, 5-50 mg, 5-75 mg, 5-100 mg, 5-250 mg, 5-500 mg, 5-750 mg, 5-1000 mg, 10-50 mg, 10-75 mg, 10-100 mg, 10-250 mg, 10-500 mg, 10-750 mg, 10-1000 mg, 25-50 mg, 25-75 mg, 25-100 mg, 25-125 mg, 25-200 mg, 25-300 mg, 25-400 mg, 25-500 mg, 25-750 mg, 25-1000 mg, 50-75 mg, 50-100 mg, 50-125 mg, 50-150 mg, 50-175 mg, 50-200 mg, 50-250 mg, 50-500 mg, 50-1000 mg, 75-100 mg, 75-125 mg, 75-150 mg, 75-250 mg, 75-500 mg, 75-1000 mg, 100-125 mg, 100-150 mg, 100-200 mg, 100-300 mg, 100-400 mg, 100-500 mg, 100-1000 mg, 125-150 mg, 125-250 mg, 125-500 mg, 125-1000 mg, 150-200 mg, 150-300 mg, 150-500 mg, 150-1000 mg, 200-300 mg, 200-400 mg, 200-500 mg, 200-750 mg, 200-1000 mg, 250-500 mg, 250-750 mg, 250-1000 mg per kilogram body weight of the subject.

According to a specific embodiment, the therapeutic effective amount of cyclophosphamide is about 25-200 mg per kilogram body weight of the subject.

As illustrated in the Examples section which follows, the present inventors have shown that administration of two doses of cyclophosphamide post transplant (on days 3 and 4 post transplant) allows for a durable engraftment and tolerance of 'mega dose' T cell depleted mismatched donor bone marrow.

According to one embodiment, cyclophosphamide is administered in a single dose.

According to one embodiment, cyclophosphamide is administered in multiple doses, e.g. in 2, 3, 4, 5 doses or more.

According to a specific embodiment, cyclophosphamide is administered in two doses.

According to one embodiment, cyclophosphamide is administered daily such as once a day or twice a day.

The dose of each cyclophosphamide administration may comprise about 5 mg, 7.5 mg, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 110 mg, 120 mg, 130 mg, 140 mg, 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, 200 mg, 210 mg, 220 mg, 230 mg, 240 mg, 250 mg, 260 mg, 270 mg, 280 mg, 290 mg, 300 mg, 350 mg, 400 mg, 450 mg or 500 mg per kilogram body weight of the subject.

According to a specific embodiment, the dose of cyclophosphamide is 50 mg per kilogram body weight of the subject.

As mentioned, cyclophosphamide is administered post transplantation. Thus, for example, cyclophosphamide may be administered to the subject 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 days or more post transplant (i.e., T+1, +2, +3, +4, +5, +6, +7, +8, +9, +10). According to a specific embodiment, cyclophosphamide is administered to the subject in two doses 3 and 4 days post transplant.

According to an embodiment, cyclophosphamide is administered prior to transplantation and post transplantation. Thus, for example, cyclophosphamide may be administered to the subject 3 days prior to transplantation (T-3) and then post transplantation (e.g. on days T+3, +4, etc.).

The number of administrations and the therapeutically effective amount of cyclophosphamide may be adjusted as needed taking into account the type of transplantation and the subject's response to the regimen. Determination of the number of administrations and the therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

In order to facilitate engraftment of the cell or tissue graft, the method may further advantageously comprise conditioning the subject with an additional immunosuppressive drug and/or immunosuppressive irradiation prior to, concomitantly with or following transplantation of the cell or tissue graft.

It will be appreciated that in situations in which the cell or tissue graft (e.g. solid organ) is transplanted prior to the T cell depleted immature hematopoietic cells, it is advisable to use general immune suppressive agents (e.g. cyclosporine A, as described in further detail below) in order to avoid organ rejection. Once the T cell depleted immature hematopoietic cells are transplanted and chimerism is achieved, the general immune suppression agents may be tapered down and subsequently stopped. In contrast in situations in which the cell or tissue graft (e.g. solid organ) is transplanted subsequent to the T cell depleted immature hematopoietic cells, after chimerism induction, the use of general immune suppression may not required.

Ample guidance for selecting and administering suitable immunosuppressive regimens for transplantation is provided in the literature of the art (for example, refer to: Kirkpatrick CH. and Rowlands DT Jr., 1992. JAMA. 268, 2952; Higgins RM. et al., 1996. Lancet 348, 1208; Suthanthiran M. and Strom TB., 1996. New Engl. J. Med. 331, 365; Midthun DE. et al., 1997. Mayo Clin Proc. 72, 175; Morrison VA. et al., 1994. Am J Med. 97, 14; Hanto DW., 1995. Annu Rev Med. 46, 381; Senderowicz AM. et al., 1997. Ann Intern Med. 126, 882; Vincenti F. et al., 1998. New Engl. J. Med. 338, 161; Dantal J. et al. 1998. Lancet 351, 623).

Thus, according to an embodiment of the present invention, the subject is conditioned under reduced intensity conditioning prior to transplantation of a cell or tissue graft.

According to an embodiment, the reduced intensity conditioning is effected for up to 2 weeks (e.g. 1-10 or 1-7 days) prior to transplantation of the cell or tissue graft.

Thus, for example, the subject may be treated with a myeloablative or non-myeloablative conditioning. Such conditioning may comprise, for example and as described in detail in the Examples section which follows, *in-vivo* T cell debulking e.g. by anti-CD4 antibody, anti-CD8 antibody, anti-CD3 (OKT3) antibodies, anti-CD52 antibodies (e.g. CAMPATH) and/or anti-thymocyte globulin (ATG) antibody (e.g. 6 days prior to transplantation at a therapeutic effective dose of about 300 μg each).

The conditioning may additionally or alternatively comprise total body irradiation (TBI), total lymphoid irradiation (TLI, i.e. exposure of all lymph nodes, the thymus, and spleen), a chemotherapeutic agent and/or an antibody immunotherapy.

Thus, according to one embodiment, the TBI comprises a single or fractionated irradiation dose within the range of 0.5-1 Gy, 0.5-1.5 Gy, 0.5-2.5 Gy, 0.5-5 Gy, 0.5-7.5 Gy, 0.5-10 Gy, 0.5-15 Gy, 1-1.5 Gy, 1-2 Gy, 1-2.5 Gy, 1-3 Gy, 1-3.5 Gy, 1-4 Gy, 1-4.5 Gy, 1-1.5 Gy, 1-7.5 Gy, 1-10 Gy, 2-3 Gy, 2-4 Gy, 2-5 Gy, 2-6 Gy, 2-7 Gy, 2-8 Gy, 2-9 Gy, 2-10 Gy, 3-4 Gy, 3-5 Gy, 3-6 Gy, 3-7 Gy, 3-8 Gy, 3-9 Gy, 3-10 Gy, 4-5 Gy, 4-6 Gy, 4-7 Gy, 4-8 Gy, 4-9 Gy, 4-10 Gy, 5-6 Gy, 5-7 Gy, 5-8 Gy, 5-9 Gy, 5-10 Gy, 6-7 Gy, 6-8 Gy, 6-9 Gy, 6-10 Gy, 7-8 Gy, 7-9 Gy, 7-10 Gy, 8-9 Gy, 8-10 Gy, 10-12 Gy or 10-15 Gy.

According to a specific embodiment, the TBI comprises a single or fractionated irradiation dose within the range of 1-3.5 Gy.

According to an embodiment, TBI treatment is administered to the subject 1-10 days (e.g. 1-3 days) prior to transplantation. According to one embodiment, the subject is conditioned once with TBI 1 or 2 days prior to transplantation.

According to a specific embodiment, the TLI comprises an irradiation dose within the range of 0.5-1 Gy, 0.5-1.5 Gy, 0.5-2.5 Gy, 0.5-5 Gy, 0.5-7.5 Gy, 0.5-10 Gy, 0.5-15 Gy, 1-1.5 Gy, 1-2 Gy, 1-2.5 Gy, 1-3 Gy, 1-3.5 Gy, 1-4 Gy, 1-4.5 Gy, 1-1.5 Gy, 1-7.5 Gy, 1-10 Gy, 2-3 Gy, 2-4 Gy, 2-5 Gy, 2-6 Gy, 2-7 Gy, 2-8 Gy, 2-9 Gy, 2-10 Gy, 3-4 Gy, 3-5 Gy, 3-6 Gy, 3-7 Gy, 3-8 Gy, 3-9 Gy, 3-10 Gy, 4-5 Gy, 4-6 Gy, 4-7 Gy, 4-8 Gy, 4-9 Gy, 4-10 Gy, 5-6 Gy, 5-7 Gy, 5-8 Gy, 5-9 Gy, 5-10 Gy, 6-7 Gy, 6-8 Gy, 6-9 Gy, 6-10 Gy, 7-8 Gy, 7-9 Gy, 7-10 Gy, 8-9 Gy, 8-10 Gy, 10-12 Gy, 10-15 Gy, 10-20 Gy, 10-30 Gy, 10-40 Gy, 10-50 Gy, 0.5-20 Gy, 0.5-30 Gy, 0.5-40 Gy or 0.5-50 Gy.

According to a specific embodiment, the TLI comprises a single or fractionated irradiation dose within the range of 1-3.5 Gy.

According to an embodiment, TLI treatment is administered to the subject 1-10 days (e.g. 1-3 days) prior to transplantation. According to one embodiment, the subject is conditioned once with TLI 2-7 days prior to transplantation.

According to one embodiment, the conditioning comprises a chemotherapeutic agent. Exemplary chemotherapeutic agents include, but are not limited to, Busulfan, Myleran, Busulfex, Fludarabine, Melphalan and Thiotepa and cyclophosphamide. The chemotherapeutic agent/s may be administered to the subject in a single dose or in several doses e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10 or more doses (e.g. daily doses) prior to transplantation. According to one embodiment, the subject is administered a

chemotherapeutic agent (e.g. Fludarabine e.g. at a dose of about 30 mg/m^2/day) for 5 consecutive days prior to transplantation (e.g. on days -7 to -3).

According to one embodiment, the conditioning comprises an antibody immunotherapy. Exemplary antibodies include, but are not limited to, an anti-CD52 antibody (e.g. Alemtuzumab sold under the brand names of e.g. Campath, MabCampath, Campath-1H and Lemtrada) and an anti-thymocyte globulin (ATG) agent [e.g. Thymoglobulin (rabbit ATG, rATG, available from Genzyme) and Atgam (equine ATG, eATG, available from Pfizer)]. Additional antibody immunotherapy may comprise anti-CD3 (OKT3), anti-CD4 or anti-CD8 agents. According to one embodiment, the antibody is administered to the subject in a single dose or in several doses e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10 or more doses (e.g. daily doses) prior to transplantation (e.g. 6 days prior to transplantation).

According to one embodiment, the subject is not treated chronically (e.g. for a prolonged period of time, e.g. for more than 10 days) with GVHD prophylaxis post transplant.

According to one embodiment, in case of relapse after hematopoietic stem cell transplantation, the subject may be further treated by donor lymphocyte infusions (DLIs). For example, the subject may be administered with graded doses of T-cells as previously described by Dazzi et al [Dazzi, Szydlo et al., Blood, (2000) 96: 2712-6] fully incorporated herein by reference.

According to one embodiment, the subject may be treated by infusion of about 0.5 - 5 x 10^4 $CD3^+$ lymphocytes per kg recipient body weight (e.g. 1 x 10^4 $CD3^+$ lymphocytes, e.g. unmanipulated $CD3^+$ lymphocytes, per kg recipient body weight) for the treatment of relapse following T cell depleted haploidentical transplantation.

According to one embodiment, a patient with early molecular and/or hematological relapse will further be treated with a first dose of about 1 x 10^4 $CD3^+$ cells per Kg recipient body weight. In the absence of GVHD, the second infusion of about 1 x 10^5 $CD3^+$ cells per kg recipient body weight will typically be given about 45 days later followed 2 months later by a third dose of about 1 x 10^6 $CD3^+$ cells per kg recipient body weight. It will be appreciated that donors typically undergo a leukoapheresis to collect lymphocytes prior to mobilization of hematopoietic cells (e.g. for transplantation). The frozen products are thawed as needed and infused quickly over

a period of 5-10 minutes. Patients exhibiting acute GVHD or who fail to demonstrate hematological engraftment typically will not receive any DLI.

According to one embodiment, a patient with relapsing B cell non-Hodgkin lymphoma will typically be further treated with rituximab (e.g. 375 mg/m^2 weekly for about 4 weeks) with DLI concomitant with the second rituximab dose.

According to one embodiment, a patient with relapsing multiple myeloma will be further treated with bortezomib (e.g. 1.3 mg/sqm on days 1, 4, 8 and 11) before starting DLI.

According to one embodiment, no post-DLI immunosuppressive agents will be used along with the present methods.

According to an aspect of the present invention, there is provided a method of treating a subject in need of a T cell depleted immature hematopoietic cell transplantation, the method comprising: (a) transplanting into a conditioned subject a dose of T cell depleted immature hematopoietic cells, wherein the T cell depleted immature hematopoietic cells comprise less than 5 x 10^5 CD3+ cells per kilogram body weight of the subject, and wherein the dose comprises at least about 5 x 10^6 CD34+ cells per kilogram body weight of the subject; and subsequently (b) administering to the subject a therapeutically effective amount of cyclophosphamide, wherein the therapeutically effective amount comprises 25-200 mg per kilogram body weight, thereby treating the subject.

According to an aspect of the present invention, there is provided a method of treating a subject in need of an immature hematopoietic cell transplantation, the method comprising: (a) conditioning a subject under a reduced intensity conditioning protocol, wherein the reduced intensity conditioning comprises a total body irradiation (TBI) and a chemotherapeutic agent; (b) transplanting into the subject a dose of T cell depleted immature hematopoietic cells, wherein the T cell depleted immature hematopoietic cells comprise less than 5 x 10^5 CD3+ cells per kilogram body weight of the subject, and wherein the dose comprises at least about 5 x 10^6 CD34+ cells per kilogram body weight of the subject; and subsequently (c) administering to the subject a therapeutically effective amount of cyclophosphamide, wherein the therapeutically effective amount comprises 25-200 mg per kilogram body weight, thereby treating the subject.

According to an aspect of the present invention, there is provided a method of inducing donor specific tolerance in a subject in need of a non-syngeneic cell or tissue graft, the method comprising: (a) transplanting into a subject a dose of T cell depleted immature hematopoietic cells obtained from a non-syngeneic donor, wherein the T cell depleted immature hematopoietic cells comprise less than 5 x 10^5 $CD3^+$ cells per kilogram body weight of the subject, and wherein the dose comprises at least about 5 x 10^6 CD34+ cells per kilogram body weight of the subject; and subsequently (b) administering to the subject a therapeutically effective amount of cyclophosphamide, wherein the therapeutically effective amount comprises 25-200 mg per kilogram body weight, thereby treating the subject.

As used herein, the term "donor specific tolerance" as used herein refers to a condition in which there is a decreased responsiveness of the recipient's cells (e.g. recipient's T cells) when they come in contact with the donor's cells (e.g. donor hematopoietic cells) as compared to the responsiveness of the recipient's cells in the absence of such a treatment method.

Tolerance induction enables transplantation of a cell or tissue graft (as described in further detail hereinabove) with reduced risk of graft rejection or GVHD.

According to one embodiment of the present invention, patients with early molecular and/or hematological relapse may receive donor lymphocyte infusions (DLI).

According to one embodiment of the present invention, DLI may comprise 1 x 10^3 - 1 x 10^6 $CD3^+$ T cell/Kg recipient body weight.

According to one embodiment, patients with early molecular and/or hematological relapse may receive a single dose or several doses (two, three, four, five or more doses) of DLI.

Thus, for example, patients with early molecular and/or hematological relapse may receive a first dose of 1 x 10^4 $CD3^+$ T cell/Kg recipient body weight. In the absence of graft versus host disease (GVHD), a second infusion of 1 x 10^5 $CD3^+$ T cell/kg recipient body weight may be given e.g. 45 days later followed e.g. 2 months later by a third dose of 1 x 10^6 $CD3^+$ T cell/kg recipient body weight.

According to one embodiment, patients with early molecular and/or hematological relapse may receive total body irradiation (TBI), total lymphoid irradiation (TLI), a chemotherapeutic agent and/or an antibody immunotherapy.

Thus, for example, patients with relapsing B cell non-Hodgkin lymphoma may receive rituximab (e.g. at a dose of 375 mg/m^2 weekly) for about 4 weeks with DLI concomitant with the second rituximab dose.

Thus, for example, patients with relapsing multiple myeloma may be treated with bortezomib (e.g. at a dose of 1.3 mg/sqm on days 1, 4, 8 and 11) before starting DLI.

As used herein the term “about” refers to ± 10 %.

The terms "comprises", "comprising", "includes", "including", “having” and their conjugates mean "including but not limited to".

The term “consisting of means “including and limited to”.

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein

interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998);

methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

Stable engraftment of HLA mismatched bone marrow following transplantation of 'mega dose' bone marrow and post transplantation cyclophosphamide

MATERIALS AND EXPERIMENTAL PROCEDURES

Animals

Mice used in these studies were 6-12 week old female mice. Balb/c-Nude (H-2d) and C3H/Hen (H-2k) were purchased from Harlan Israel (Rehovot, Israel). All mice were kept in small cages (5 animals in each cage) and fed sterile food and acid water.

These studies were approved by the Weizmann Institute of Science, Institutional Animal Care and Use Committee.

Transplantation protocol

Low (5 x 10^6) and high dose (25 x 10^6) Balb/c-Nude BM cells (providing a source of BM depleted of T cells) were transplanted into allogeneic recipients (C3H/Hen) on day 0 following *in-vivo* T cell debulking (TCD) with anti-CD4 (clone GK1.5) and anti-CD8 (clone 53.6.72) antibodies (300 μg each; Bio X Cell, NH, USA) delivered on day -6, and exposure to 2.0 Gy total body irradiation (TBI) on day -1. High dose Cyclophosphamide (CY, 100 mg/kg, Baxter Oncology, Germany) was administered on days +3 and +4 post transplant and donor type chimerism was evaluated 35 and 95 days post transplant using fluorescein anti-host and donor H-2 antibodies (e.g. FITC labeled anti-H-$2D^d$ antibody specific for donor type cells and PE labeled anti-H-$2K^k$ antibody specific for host type cells).

Skin graft protocol

Donor (Balb/c) and 3rd party (C57BL/6) skin grafts were transplanted to the mixed chimeric recipients as described above [i.e. to those mice which were previously transplanted with mega dose (25 x 10^6) T cell depleted BM and were treated with high dose CY] and to the recipient mice that were inoculated with a regular (5 x 10^6) T cell depleted BM cell dose and were treated with high dose CY.

RESULTS

To test the potential synergy between 'mega dose' T cell depleted bone marrow transplant (BMT) and high dose cyclophosphamide (CY) post transplant, after reduced intensity conditioning (RIC) of the recipient mice, the following experiments were carried out.

Recipient mice (C3H/Hen) were treated with a conditioning protocol prior to transplantation of a T cell depleted bone marrow transplant. Specifically, mice were *in-vivo* treated with T cell debulking (TCD) using anti-CD4 and anti-CD8 antibodies delivered on day -6 and by exposure to 2.0 Gy total body irradiation (TBI) on day -1. Next, low (5 x 10^6) or high dose (25 x 10^6) Balb/c-Nude BM cells (providing a source of BM depleted of T cells, as Nude mice have miniscule numbers of mature T cells) were transplanted into allogeneic recipients (C3H/Hen) on day 0. High dose cyclophosphamide (CY, 100 mg/kg) was administered on days +3 and +4 post

transplant. Evaluation of bone marrow cell engraftment was evaluated by donor type chimerism at 35 and 95 days post transplant.

As shown in Figures 1A-B and Figures 2A-B, chimerism analysis on day 35 and day 95 revealed that none of the control mice (conditioned with TCD, 2 Gy TBI and optionally CY, but did not receive BM) expressed donor type chimerism. Similarly, none of the BM mice recipients that were transplanted with a regular dose of 5 x 10^6 T cell depleted BM, in the presence or absence of cyclophosphamide treatment, expressed donor type chimerism. However when the dose of T cell depleted BM was increased to 25 x 10^6 cells, durable mix chimerism was achieved in 4 out of 7 mice that were also treated with cyclophosphamide on days +3 and +4 post transplant (see Figures 1A-B and Figure 2C).

Further follow-up of these recipient mice at 180 and 225 days post transplant revealed that the chimerism induced was stable and durable (Figure 3). As illustrated in Figure 3, the number of donor type chimeric recipients and the level of donor chimerism remained unchanged 225 days post transplant, suggesting that tolerance has been achieved.

Tolerance induction was measured by transplantation of donor (Balb/c) and 3rd party (C57BL/6) skin grafts to the mixed chimeric recipients that were transplanted with mega dose (25 x 10^6) T cell depleted BM and were treated with high dose CY (as described above), in comparison to the recipients that were inoculated with a regular (5 x 10^6) T cell depleted BM cell dose (as described above).

As shown in Figures 4A-B, three out of 4 chimeric mice that were transplanted with 25 x 10^6 T cell depleted BM accepted the donor graft and rejected the 3rd party skin grafts. In contrast, recipient mice that were inoculated with 5 x 10^6 T cell depleted BM cells and CY rejected both the donor and 3rd party skin grafts (Figure 4A).

These results illustrate that the combination of mega dose T cell depleted BM and high dose Cyclophosphamide treatment allows the successful engraftment of hematopoietic stem cells, under reduced intensity conditioning, along with tolerance induction.

Encouraged by these results a set of calibration experiments were initiated in order to determine the optimal irradiation and Cyclophosphamide dose to improve chimerism induction by this approach.

EXAMPLE 2

The effect of different doses of total body irradiation (TBI) on chimerism

MATERIALS AND EXPERIMENTAL PROCEDURES

Animals

As described in Example 1, hereinabove.

Transplantation protocol

High dose (25 x 10^6) Balb/c-Nude BM cells (providing a source of BM depleted of T cells) were transplanted into allogeneic recipients (C3H/Hen) on day 0 following *in-vivo* T cell debulking (TCD) with anti-CD4 (clone GK1.5) and anti-CD8 (clone 53.6.72) antibodies (300 µg each; Bio X Cell, NH, USA) delivered on day -6, and exposure to different doses of irradiation ranging from 1 to 3.5 Gy TBI on day -1. High dose Cyclophosphamide (CY, 100 mg/kg, Baxter Oncology, Germany) was administered on days +3 and +4 post transplant and donor type chimerism was evaluated 30 days post transplant using fluorescein anti-host and donor H-2 antibodies (e.g. FITC labeled anti-H-$2D^d$ antibody specific for donor type cells and PE labeled anti-H-$2K^k$ antibody specific for host type cells).

RESULTS

In this experiment, the minimal irradiation dose was defined. 'Mega dose' (25 x 10^6) Balb/c-Nude T cell depleted BM was transplanted into 5 groups of allogeneic recipients (C3H/Hen) on day 0 following T cell debulking (with anti-CD4 and anti-CD8 antibodies) on day -6, and different doses of irradiation (ranging from 1 to 3.5 Gy TBI) on day -1. High dose Cyclophosphamide (CY) was administered on days +3 and +4 post transplant and donor type chimerism was evaluated at 30 days post transplant.

As can be seen in Figure 5, all the recipient mice that were irradiated with 2.5, 3 or 3.5 Gy TBI (6/6) were chimeric, exhibiting donor type chimerism ranging between 58 - 83 %. Similarly, 87 % (13/15) of the mice treated with 2 Gy TBI exhibited donor type chimerism ranging between 56 - 85 %.

Further reduction of the irradiation dose to 1.0 Gy caused a small reduction in the percentage of chimeric mice, namely 83 % (5/6), however the donor type chimerism range was significantly reduced to 14.5 - 58 %.

EXAMPLE 3

The effect of different Cyclophosphamide (CY) doses on chimerism

MATERIALS AND EXPERIMENTAL PROCEDURES

Animals

As described in Example 1, hereinabove.

Transplantation protocol

High dose (25 x 10^6) Balb/c-Nude BM cells (providing a source of BM depleted of T cells) were transplanted into allogeneic recipients (C3H/Hen) on day 0 following *in-vivo* T cell debulking (TCD) with anti-CD4 (clone GK1.5) and anti-CD8 (clone 53.6.72) antibodies (300 μg each; Bio X Cell, NH, USA) delivered on day -6, and exposure to 2.0 Gy total body irradiation (TBI) on day -1. Different doses of Cyclophosphamide (CY, 100 mg/kg, 125 mg/kg or 150 mg/kg, Baxter Oncology, Germany) were administered on days +3 and +4 post transplant and donor type chimerism was evaluated 30 days post transplant using fluorescein anti-host and donor H-2 antibodies (e.g. FlTC labeled anti-H-$2D^d$ antibody specific for donor type cells and PE labeled anti-H-$2K^k$ antibody specific for host type cells).

RESULTS

In this experiment, the optimal dose of CY post transplant was defined. 'Mega dose' (25 x 10^6) Balb/c-Nude BM cells were transplanted into 3 groups of allogeneic recipients (C3H/Hen) on day 0 following T cell debulking (TCD) with anti-CD4 and anti-CD8 antibodies on day -6, and 2 Gy TBI on day -1. Different doses of Cyclophosphamide (CY), 100 mg/kg, 125 mg/kg or 150 mg/kg, were administered on days +3 and +4 post transplant and donor type chimerism was performed 30 days post transplant.

As can be seen in Figure 6, increasing CY dose to 125 mg/kg or 150 mg/kg did not provide a significant enhancement of chimerism. Thus, the recipient mice that were treated with 100 mg/kg, 125 mg/kg or 150 mg/kg CY exhibited an average of 57.5 ± 25.8, 66.5 ± 20.6 or 67.4 ± 27.4 donor type chimerism, respectively. No statistical significance was found when the recipients treated with 100 mg/kg were compared to those treated with 125 mg/kg or 150 mg/kg (P=0.5 and p=0.469 respectively).

EXAMPLE 4

CD8$^+$ Non-T cells are not important for attaining chimerism by combining 'mega dose' T cell depleted BM with CY post transplant

MATERIALS AND EXPERIMENTAL PROCEDURES

Animals

As described in Example 1, hereinabove.

Transplantation protocol

High dose (25 x 10^6) of CD8 depleted and non-depleted Balb/c-Nude BM cells were transplanted into 2 cohorts of allogeneic recipients (C3H/Hen) on day 0 following *in-vivo* T cell debulking (TCD) with anti-CD4 (clone GK1.5) and anti-CD8 (clone 53.6.72) antibodies (300 μg each; Bio X Cell, NH, USA) delivered on day -6, and exposure to 2.0 Gy total body irradiation (TBI) on day -1. High dose Cyclophosphamide (CY, 100 mg/kg, Baxter Oncology, Germany) was administered on days +3 and +4 post transplant and donor type chimerism was evaluated 30 days post transplant using fluorescein anti-host and donor H-2 antibodies (e.g. FITC labeled anti-H-2D^d antibody specific for donor type cells and PE labeled anti-H-2K^k antibody specific for host type cells).

The BM source in these experiments was Balb/c-Nude mice. Moreover, the transplanted mice in these experiments were athymic and as such they lacked T cells. However in order to refute the possibility that the effect was a contribution of residual non-T CD8 cells, the BM preparation from Balb/c-Nude mice was negatively sorted for CD8 cells using a cell sorting system (e.g. anti-CD8 magnetic beads or FACS sorter).

RESULTS

As Ildstad et al. previously taught that a subset of CD8$^+$ TCR$^-$ BM cells are critical for achieving donor type chimerism [Fugier-Vivier IJ et al., *J Exp Med* (2005) 201:373-383; Grimes HL et al., *Exp Hematol.* (2004) 32:946-954; Huang Y et al., *Blood* (2011) 117:2494-2505; Kaufman CL et al., *Blood* (1994) 84:2436-2446; Leventhal J et al., *BMC Med* (2012) 10:48; Leventhal J et al., *Sci Transl Med.* (2012) 4:124ra128], the present inventors depleted residual CD8$^+$ cells from the Balb/c–Nude 'mega dose' BM preparation, and measured chimerism induction compared to control non-CD8$^+$ depleted Nude BM cells.

As can be seen in Figure 7, depletion of $CD8^+$ T cells from the BM preparation did not have any adverse impact on the level of chimerism achieved when combing 'mega dose' T cell depleted BM cells with post transplant CY.

EXAMPLE 5

Clinical protocol

STUDY DESIGN

This is a prospective, observational, phase I/II multicenter study. Ten patients with hematological disorders will be enrolled over a one year period.

The primary endpoint of the study is engraftment and 10 evaluable patients (i.e. patients surviving beyond day 28) will be entered. An acceptable primary graft failure or rejection rate is approximately 10 %.

Study duration

The primary analysis will be conducted using 6 and 12 months follow-up data. Patients will be followed-up until 48 months after transplantation.

Definitions

Stable sustained engraftment is defined as neutrophils, more than 1000/μl for three consecutive days, and platelets, more than 20000/μl for three consecutive days, without transfusion.

Graft rejection is defined as rapid decline of neutrophils, less than 100/μl after documented neutrophil engraftment, with or without increase of lymphocytes.

Graft failure is defined as failure to reach more than 1000/μl neutrophils for three consecutive days and more than 20000/μl platelets for three consecutive days without transfusion at day +28.

The secondary endpoint of the study is the incidence of grade II-IV acute GVHD. An acceptable incidence of grade II-IV acute GVHD is approximately 10 %.

For acute GVHD grading criteria is indicated in Tables 1A-B, below.

Table 1A: Clinical staging of acute GVHD

Stage	SKIN	LIVER	GUT
+	Rash more than 25 %	Bilirubin = 2-3 mg/dl	Diarrhea 500-1000 ml
++	Rash 25-50 %	Bilirubin = 3-6 mg/dl	Diarrhea 1000-1500 ml
+++	Generalized erythroderma	Bilirubin = 6-15 mg/dl	Diarrhea more than 1500 ml
++++	Desquamation and bullae	Bilirubin more than 15 mg/dl	Pain or ileus

Table 1B: Clinical grading of acute GVHD

GRADE	SKIN	LIVER	GUT	Functional impairment
0 none	0	0	0	0
I mild	+ to ++	0	0	0
II moderate	+ to +++	+	+	+
III severe	++ to +++	++ to +++	++ to +++	++
IV / life threatening	++ to ++++	++ to ++++	++ to ++++	+++

Statistical considerations

The time intervals for engraftment, survival, disease-free survival, relapse rate and risk of transplant-related mortality will be calculated from the day of stem cell transplantation. Actuarial curves will be calculated according to the Kaplan-Meier method.

ELIGIBILITY CRITERIA

Inclusion Criteria - Patient

- Age - more or equal to 18 and less or equal to 70 years old
- CLL patients with refractoriness to fludarabine or other chemotherapy due to the p53 loss by 17p deletion and/or TP53 mutation
- Follicular lymphoma with either unfavorable cytogenetics such as complex karyotype, del17p, del 6q23-26, mutations in TP53, minus 1p

- Hodgkin's Lymphoma relapsed after autologous transplantation, not eligible for immunotherapy with anti-CD30
- Multiple myeloma relapsing after autologous transplantation, with unfavorable cytogenetics in either partial or complete remission
- Severe Aplastic Anemia relapsing after immunotherapy
- Absence of fully HLA-matched or one locus HLA-mismatched family donor
- Absence of matched unrelated donor or ineligibility for donor search in the donor registry (IBMDR)
- Presence of haploidentical family donor and a back-up of patient autologous stem cells
- Stable clinical conditions and life expectancy of more than 12 weeks
- Karnofsky - more than 70 %
- Written informed consent

Pre-treatment evaluation

- complete clinical history and examination and determination of performance status and body surface area.
- complete blood count
- blood group, red blood cells subgroups, anti-A and/or anti-B agglutinin titration
- creatinine clearance, uric acid, ferritin, LDH, beta 2 microglobulin, protein electrophoresis, SGOT, SGPT, urine test, blood glucose, blood nitrogen, immunoglobulin levels, Coombs tests.
- pregnancy test
- HIV-ab, HBsAg, HBVDNA, HCV-ab, HCVRNA, CMV-ab, Toxoplasma-ab, HSVab
- ECG and measurement of ejection fraction by ultrasound or scintigraphic test.
- chest X ray.
- lung CT scan, brain CT scan, maxillary sinus CT scan.
- dental X ray and examination.

- biopsy and bone marrow aspirate for morphologic and cytogenetic analysis, search for a molecular marker (if not known) and FACS analysis (according to underlying disease).
- neurologic examination and lumbar puncture in patient at risk.
- radiologic scan (CT, NMR) of the known disease localization.
- complete serologic and molecular HLA typing, ML cultures and cytotoxicity test with the selected donors.
- cytotoxic anti HLA antibodies.
- Abdominal echography

Exclusion criteria - Patient

- History of central nervous system disease localization
- Positivity for HIV, HCV, HCVRNA, HBsAg, HBVDNA
- Active and documented pneumonia of any kind, fungal tissue infection, viral positive cultures of respiratory secretion or blood
- bilirubin of more than 2 times normal
- blood creatinine clearance less than 50 ml/min
- DLCO less than 50 % of the predicted value
- ejection fraction less than 45 % (or myocardial stroke in the last year)
- pregnancy or lactation
- psychiatric disorders

Eligibility Criteria - Donor

- Absence of hematopoietic or marrow function related disease that interferes with the collection of sufficient numbers of normal progenitor cells.
- Absence of any medical condition that would pose a serious health risk by undergoing peripheral blood stem cell harvest
- Negative HIV, HTLV-1 tests
- Any healthy family member will be considered for hematopoietic stem cell donation. Selection of a donor will be based on typing of HLA-A, B, C, DR loci to be carried out on the recipient, siblings, parents and possibly other family members such as aunts, uncles and cousins. A prospective related donor must be at least genotypically HLA-A, B, C, DR haploidentical to the patient, but can differ for 2-3 HLA alleles on the unshared haplotype.

- Donor will be selected preferentially on the basis of the donor-versus-recipient NK alloreactivity.

Donor Evaluation

- Complete history, physical examination and examination of physical veins by the pheresis service for determination of suitability for pheresis via peripheral veins.
- Blood tests: WBC, PLT, Hb, PT, PTT, total protein, albumin, electrolytes, glucose, SGOT/SGPT, alkaline phosphatase, bilirubin, LDH, acid uric, creatinine.
- CMV, EBV, HSV, VZV, Hepatitis B + C, HIV, Toxoplasma serology.
- Complete red blood cell typing
- Serology for Syphilis, CMV, EBV, HSV, VZV, Hepatitis B + C, HTLV-1, HIV, Toxoplasmosis.
- Transfusion transmitted disease testing must be performed between 30 and 7 days prior to stem cell collection
- Chest X-ray
- EKG
- VNTR analysis by PCR
- Donors will be prioritized on the basis of younger age, better health, and being CMV-negative for CMV-negative recipients.

Exclusion Criteria - Donor

- A positive HIV or HTLV-1 test or evidence of active/persistent viral hepatitis infection will exclude the donor from participation in this study.
- Presence of any medical condition that would pose a serious health risk by undergoing peripheral blood stem cell harvest (i.e. insulin-dependent diabetes, cardiovascular disorders, chronic inflammatory diseases).

TREATMENT PROCEDURES

Mobilization of donor HSC and Graft processing.

Patients are required to have a family donor (aged 18 to 60 yrs), willing and capable of donating filgrastim/lenogastrim-stimulated peripheral blood hematopoietic cells. Donors will be screened according to Blood Bank general rules. It is advisable to

perform an exercise EKG testing in donors above 50 yrs of age. Normal donors will receive filgrastim or lenogastrim 5 mcg/kg subcutaneously every 12 hours; on day 5 the leukapheresis will be started. Filgrastim/Lenogastrim dosage will be adjusted to maintain white blood cells below 60 x 10^9/L. On the 4th day of filgrastim/lenograstim treatment, if the circulating CD34+ cell count is more than 40/μL, the donor will start leukapheresis. Daily leukapheresis will be continued for a planned 3 days, with a maximum of 4 days, to collect a target cell dose of more than 10 x 10^6 CD34+ cells/kg. If the target is reached early, collection can continue for 3 total days in order to give the largest possible dose. If the donor does not tolerate the procedure in any of its component parts, an alternative donor may be used if available. If a site is unable to collect more than 10 x 10^6 CD34+ cells/kg from an appropriate donor, patients may not proceed on study. PBPCs will be depleted of donor T and B cells by selection of CD3+ and/or CD19+ cells using a cell sorting system (e.g. anti-CD3/19 magnetic beads or FACS sorter). Target value of CD34-positive cells will be at least 10 x 10^6/kg of the recipient body weight (b.w.).

The apheresis will be performed through the antecubital veins.

Table 2: Conditioning regimen

day -7	Fludarabine 30 mg/sqm
day -6	Fludarabine 30 mg/sqm
day -5	Fludarabine 30 mg/sqm
day -4	Fludarabine 30 mg/sqm
day -3	Fludarabine 30 mg/sqm
day -2	TBI 2 Gy single fraction
day -1	Rest
day 0	Graft
day +1	Rest
day +2	Rest
day +3	CY 50 mg/kg
day +4	CY 50 mg/kg

As described in Table 2, above, fludarabine will be administered intravenously daily on 5 sequential days, -7, -6, -5, -4, and –3, at a dose of 30 mg/m^2. Each dose will be infused over 30 minutes. TBI 200 cGy will be given on day –1 in a single fraction.

On day 0, $CD3^-/CD19^-$ immunoselected HSCs will be thawed, washed and infused through a central access.

CY will be administered intravenously in one hour on days +3 and +4 post-transplantation at 50 mg/kg/day.

Special management orders

a. a double-lumen central venous line will be placed before conditioning regimen;

b. for urate prophylaxis allopurinol 300 mg per os will be given;

c. antiemetic therapy will be given according to single center guidelines;

d. transfusion of filtered and irradiated blood products. Keep hemoglobin level more than 8 g/L and platelets more than 15000/ μL in absence of fever or bleeding signs;

Patient monitoring during treatment

a. daily full blood count and differential

b. serum creatinine, Na+, K+, Ca++, bilirubin daily during chemotherapy and hyper-hydration

c. liver function tests, albumin, coagulation tests with antitrombin III, cytomegalovirus antigenemia and PCR twice a week.

d. surveillance cultures according to center guidelines

TOXICITY EVALUATION

Toxicity will be evaluated according to WHO criteria, as indicated in Table 3, below.

Table 3: WHO toxicity criteria

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Hematological					
Hemoglobin	≥ 11.0 g/dl ≥ 6.8 mmol/l	9.5-10.9 g/dl 5.6-6.7 mmol/l	8.0-9.4 g/dl 4.9-5.6 mmol/l	6.5-7.9 g/dl 4.0-4.9 mmol/l	< 6.5 g/dl < 4.0 mmol/l
Leukocytes (1000/mm)	≥ 4.0	3.0-3.9	2.0-2.9	1.0-1.9	< 1.0
Granulocytes (1000/mm)	≥ 2.0	1.5-1.9	1.0-1.4	0.5-0.9	< 0.5
Platelets (1000/mm)	≥ 100	75-99	50-74	25-49	< 25

Hemorrhage	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 phosphotase	≤ 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	Hemorrhagic dehydration
Renal					
Blood urea or 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
Hematuria	No change	Microscopic	Gross	Gross + clots	Obstructive uropathy
Pulmonary	No change	Mild symptoms	Exertional dyspnoea	Dyspnoea at rest	Complete bed rest required
Fever with drug	None	Fever < 38 °C	Fever 38-40 °C	Fever > 40 °C	Fever with hypotension
Allergic	No change	Oedema	Bronchospasm : no parenteral therapy needed	Bronchospasm: parenteral therapy required	Anaphylaxis
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.

SUPPORTIVE CARE

Monitoring and treatment of bacterial and fungal infections

Patients are cared for in isolation rooms with laminar airflow or high-efficiency air-particulate filtration. Liposomal Amphotericin is given at 1 mg/kg/day from day -5 to engraftment as antifungal prophylaxis. Bacterial infections are monitored by swabs and blood cultures weekly. Intravenous antibiotic therapy is started on the basis of clinical signs of infection (fever of unknown origin) or positive blood cultures. If the patient is still febrile after 72 hours, empiric antifungal therapy is started using either L-AMB 3 mg/kg/day or Voriconazole 8 mg/kg/day i.v. Vancomycin is added after an additional 72 hours of fever, or in the presence of Gram+ sepsis, or positive blood culture.

Prophylaxis, monitoring and treatment of cytomegalovirus infections

In recipients who are seropositive for CMV antibody, CMV prophylaxis consists of ganciclovir (10 mg/kg/day) between the tenth and second day before stem cell

infusion. Ganciclovir is reintroduced as preemptive therapy from day +21 until day +360. CMV antigenemia/PCR is determined weekly in blood samples. If CMV antigenemia/PCR develops, patients will treated with ganciclovir (10 mg/kg/day) or foscarnet (180 mg/kg/day).

The blood products are irradiated (30 Gy) before transfusion.

Post-Transplant Laboratory Evaluation:

1. Daily complete hemograms until granulocytes and platelets are self-sustaining, then three times/week until discharge; at least every week post discharge to day 100 and then every 2 weeks to 12 months.

2. Screening profile with liver and renal function tests twice weekly for the first 30 days, then weekly to discharge; more frequently if clinically indicated.

3. Bone marrow aspirates for morphology analysis of chimerism by FISH (sex-mismatched grafts) or cytogenetics will be done at approximately 1, 3, 6, 12 months, and every 4 months thereafter for approximately 3 years. Additional analysis will be done as clinically indicated. Patients with CML will be also monitored for bcr/abl evidence of recurrence

4. Immunological reconstitution will be monitored by in vitro assays, including phenotypic analysis of circulating lymphocytes, assessment of natural killer and lymphokine activated killer cell function, lymphocyte transformation responses to T-cell and B-cell mitogens and immunoglobulin levels.

Follow-up

Until day +90 complete blood counts, antigenemia and PCR for CMV, reactive protein C, complete liver and renal function will be assess twice a week.

Every two weeks until +90 peripheral blood phenotype (CD3, CD4, CD8, CD19, CD56, CD57, HLADR), chest Xray.

Every two weeks from +90 till +180:

complete blood counts, antigenemia and PCR for CMV, reactive protein C, complete liver and renal function.

Monthly:

immunoglobulin levels, protein electrophoresis,

after +90 peripheral blood phenotype (CD3, CD4, CD8, CD19, CD56, CD57, HLADR), chest Xray.

after + 180 complete blood counts, antigenemia and PCR for CMV, reactive protein C, complete liver and renal function.

Complete restaging of disease will be performed 2, 4, 6, 8, 12, 18 and 24 months after transplantation then annually, this will include assessment of donor chimerism by PCR analysis of HLA on peripheral blood and bone marrow cells.

For Performance Status grading criteria see Table 4, below.

Table 4: Karnofsky Performance scale

FUNCTIONAL STATUS	*RATING*	*GROUP SCORES*
Normal. No complaints. No evidence of disease. Able to carry on normal activity. Minor signs or symptoms of disease. Normal activity with effort. Some signs or symptoms of disease.	100 90 80	Rehabilitated (80+)
Cares for self. Unable to carry on normal activity or do active work.	70	Self-care only (70-90)
Requires occasional assistance, but able to care for most needs. Requires considerable assistance and frequent medical care. Disabled. Requires special care and assistance.	60 50 40	Requires Caretaker (40-69)
Severely disabled. Hospitalisation is indicated, although death is not imminent. Very sick. Hospitalisation necessary. Moribund. Fatal processes progressing. Dead.	30 20 10 0	Requires institutionalisation (1-39)

PROGRAMMED INFUSIONS of DONOR LYMPHOCYTES

Donor lymphocyte infusions (DLIs) are effective to treat relapses after allogeneic HSCT. Nevertheless, the success of DLI has been limited to some extent by the morbidity and mortality associated with GVHD. Graded doses of T-cells are less likely to produce GVHD than a single large infusion and appear to be as effective to induce remission [Dazzi, Szydlo et al., Blood, (2000) 96: 2712-6]. A recent dose finding study has shown that 1 x 10^4 unmanipulated $CD3^+$ lymphocyte/kg recipient b.w. can be safely infused in patients who have received a T cell depleted haploidentical transplantation [Lewalle P. et al. Bone Marrow Transplant (2002) 29 (suppl 2): S26, 0164a].

Patients with early molecular and/or hematological relapse will receive a first dose of 1 x 10^4 $CD3^+$ cell/Kg recipient b.w.; in the absence of GvHD, the second infusion of 1 x 10^5 $CD3^+$ cell/kg will be given 45 days later followed 2 months later by a third dose of 1 x 10^6 $CD3^+$ cell/kg. Donors will undergo a leukoapheresis to collect lymphocytes prior to mobilization of hematopoietic cells because it has been shown that G-CSF has an immune-modulatory effect on some T lymphocyte subsets, decreasing their responsiveness to allogeneic stimuli. The frozen products will be thawed and infused quickly over a period of 5-10 minutes. Patients with acute GvHD or who fail to demonstrate hematological engraftment will not receive any DLI.

Patients with relapsing B cell non-Hodgkin lymphoma will receive rituximab 375 mg/m^2 weekly for 4 weeks with DLI concomitant with the second rituximab dose. Patients with relapsing multiple myeloma will be treated with bortezomib (1.3 mg/sqm on days 1, 4, 8 and 11) before starting DLI.

No post-DLI immunosuppressive agents will be used

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. Use of a dose of T cell depleted immature hematopoietic cells from a non-syngeneic donor and a therapeutically effective amount of cyclophosphamide in the manufacture of a medicament for combination therapy for reducing graft rejection and/or graft versus host disease (GVHD) and/or for inducing donor specific tolerance in a conditioned subject in need of a non-syngeneic cell or tissue graft,

wherein said conditioned subject has been conditioned under a reduced intensity conditioning protocol comprising a total body irradiation (TBI) or a total lymphoid irradiation (TLI) at an irradiation dose within the range of 0.5-5 Gy,

wherein said T cell depleted immature hematopoietic cells comprise less than 5 x 10^5 $CD3^+$ cells per kilogram body weight, and wherein said dose comprises at least 5 x 10^6 CD34+ cells per kilogram body weight,

wherein said therapeutically effective amount of cyclophosphamide comprises 25-200 mg per kilogram body weight, and wherein said cyclophosphamide is to be administered to the subject following transplantation of the T cell depleted immature hematopoietic cells, and further wherein said subject is not treated with GVHD prophylaxis for more than 10 days post-transplant.

2. The use of claim 1, wherein said reduced intensity conditioning protocol is effected 1-10 days.

3. The use of claim 1, wherein said reduced intensity conditioning comprises a non-myeloablative conditioning protocol.

4. The use of claim 3, wherein said non-myeloablative conditioning protocol comprises a chemotherapeutic agent and/or an antibody immunotherapy.

5. The use of claim 1, wherein said TBI comprises a single or fractionated irradiation dose within the range of 1-3.5 Gy.

6. The use of claim 1, wherein said TLI comprises a single or fractionated irradiation dose within the range of 1-3.5 Gy.

7. The use of claim 4, wherein said chemotherapeutic agent comprises at least one of Busulfan, Fludarabine, Melphalan and Thiotepa.

8. The use of claim 4, wherein said antibody comprises at least one of an anti-CD52 antibody, an anti-thymocyte globulin (ATG) antibody and anti-CD3 (OKT3) antibody.

9. The use of claim 1, wherein said conditioned subject has been conditioned with *in-vivo* T cell debulking.

10. The use of claim 9, wherein said *in-vivo* T cell debulking is effected 4-7 days.

11. The use of claim 9, wherein said *in-vivo* T cell debulking is effected by antibodies.

12. The use of claim 11, wherein said antibodies comprise an anti-CD8 antibody, an anti-CD4 antibody or both.

13. The use of claim 11, wherein said antibodies comprise anti-thymocyte globulin (ATG) antibodies, an anti-thymocyte globulin (ATG) antibody and anti-CD3 (OKT3) antibody.

14. The use of any one of claims 1-13, wherein a dose of said T cell depleted immature hematopoietic cells comprises 5 - 40 x 10^6 CD34+ cells per kilogram body weight of the subject.

15. The use of claim 14, wherein a dose of said T cell depleted immature hematopoietic cells comprises at least 10 x 10^6 CD34+ cells per kilogram body weight of the subject.

16. The use of any one of claims 1-15, wherein said T cell depleted immature hematopoietic cells are selected from the group consisting of T cell depleted bone

marrow cells, T cell depleted G-CSF mobilized peripheral blood progenitor cells, T cell depleted cord blood, purified CD34+ cells attained by positive selection from bone marrow and/or from G-CSF mobilized peripheral blood progenitor cells, and ex-vivo expanded $CD34^+$ cells.

17. The use of any one of claims 1-16, wherein said T cell depleted immature hematopoietic cells comprise less than 1 x 10^6 $CD8^+$ $TCR\alpha/\beta^-$ cells per kilogram body weight.

18. The use of any one of claims 1-17, wherein said T cell depleted immature hematopoietic cells are obtained by T cell debulking.

19. The use of claim 18, wherein said T cell debulking is effected by antibodies.

20. The use of claim 19, wherein said antibodies are selected from the group consisting of an anti-CD8 antibody, an anti-CD4 antibody, an anti-CD3 antibody, an anti-CD2 antibody and an anti-TCRα/β antibody.

21. The use of any one of claims 1-20, wherein said immature hematopoietic cells are treated by B cell debulking.

22. The use of claim 21, wherein said B cell debulking is effected by an anti-CD19 antibody or by anti-CD20 antibody.

23. The use of claim 1, wherein said non-syngeneic donor is allogeneic with respect to the subject.

24. The use of claim 23, wherein said allogeneic donor is selected from the group consisting of an HLA matched sibling, an HLA matched unrelated donor, an HLA haploidentical related donor and a donor displaying one or more disparate HLA determinants.

25. The use of any one of claims 1-24, wherein the subject is a human subject.

26. The use of any one of claims 1-25, wherein said concentration of said cyclophosphamide is 100 - 200 mg per kg body weight of the subject.

27. The use of claim 26, wherein said concentration of said cyclophosphamide is about 100 mg per kg body weight of the subject.

28. The use of any one of claims 1-27, wherein said cyclophosphamide is effected in two doses.

29. The use of claim 28, wherein each of said two doses comprises a concentration of about 50 mg per kg body weight of the subject.

30. The use of any one of claims 1-29, wherein said cyclophosphamide is to be administered to the subject in two doses 3 and 4 days post-transplant.

31. The use of any one of claims 1-30, wherein said subject has a malignant disease.

32. The use of claim 31, wherein said malignant disease is a hematopoietic cancer.

33. The use of claim 32, wherein said hematopoietic cancer comprises a leukemia or lymphoma.

34. The use of claim 32, wherein said hematopoietic cancer is selected from the group consisting of acute lymphoblastic leukemia (ALL), acute myelocytic leukemia (AML), acute nonlymphoblastic leukemia (ANLL), Chronic lymphocytic leukemia (CLL), chronic myelocytic leukemia (CML), Hodgkin's Lymphoma, non- Hodgkin's Lymphoma, Extranodal natural killer/T-cell lymphoma, Cutaneous T-cell lymphoma, Enteropathy type T-cell lymphoma, Angioimmunoblastic T-cell lymphoma, Anaplastic

large T/null-cell lymphoma, Subcutaneous panniculitis-like T-cell lymphoma, Unspecified T-cell lymphoma, Diffuse large B-cell lymphoma, B-cell chronic lymphocytic leukemia (B-CLL)/chronic lymphoid leukemia (CLL), Chronic lymphocytic leukemia/small lymphocytic lymphoma, Extranodal marginal zone B-cell lymphomas - mucosa-associated lymphoid tissue lymphomas, Follicular lymphoma, Mantle cell lymphoma, Nodal marginal zone B-cell lymphoma, Burkitt lymphoma, Hairy cell leukemia, Primary central nervous system lymphoma, Splenic marginal zone B-cell lymphoma, Lymphoplasmocytic lymphoma, Primary mediastinal B-cell lymphoma and multiple myeloma.

35. The use of claim 30, wherein said subject has disease selected from the group consisting of a genetic disease or disorder, an autoimmune disease, a metabolic disorder, a hematopoietic disease, a cardiovascular disease, a rheumatoid disease, a glandular disease, a gastrointestinal disease, a cutaneous disease, a hepatic disease, a neurological disease, a muscular disease, a nephric disease, a connective tissue disease, a systemic disease and a disease related to reproduction.

36. The use of claim 35, wherein said subject has disease selected from the group consisting of a sickle cells disease, a congenital neutropenia, a thrombocytopenia, an aplastic anemia, a myelodysplastic syndrome, a monosomy 7, an osteopetrosis, a Gaucher's disease, a Hurler's disease, a metachromatic leukodystrophy, an adrenal leukodystrophy, a thalassemia, a congenital or genetically-determined hematopoietic abnormality, lupus, autoimmune hepatitis, celiac disease, type I diabetes mellitus, Grave's disease, Guillain-Barr syndrome, Myasthenia gravis, Rheumatoid arthritis, scleroderma and psoriasis.

37. The use of any one of claims 1-36, wherein said non-syngeneic cell or tissue graft comprises immature hematopoietic cells.

38. The use of any one of claims 1-36, wherein said non-syngeneic cell or tissue graft is selected from the group consisting of a liver, a pancreas, a spleen, a kidney, a heart, a lung, a skin, an intestine and a lymphoid/hematopoietic tissue or organ.

39. The use of claim 38, wherein said cell or tissue graft comprises a co-transplantation of several organs.

40. The use of any one of claims 37-39, wherein said cell or tissue graft and said T cell depleted immature hematopoietic cells are obtained from the same donor.