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(54) **Anti-harmadik fél központi memória T-sejtek alkalmazása leukémia/limfóma-ellenes kezeléshez**

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(54) **USE OF ANTI THIRD PARTY CENTRAL MEMORY T CELLS FOR ANTI-LEUKEMIA/LYMPHOMA TREATMENT**

ZENTRALE ANTI-DRITTE-T-GEDÄCHTNISZELLEN, UND DEREN VERWENDUNG IN EINER 'ANTI-LEUKÄMIE/LYMPHOMA' BEHANDLUNG

LYMPHOCYTES T À MÉMOIRE CENTRALE ANTI-TIERS, ET UTILISATION DE CEUX-CI DANS UN TRAITEMENT & xA;'ANTI-LEUKEMIA/LYMPHOMA'

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Description

[0001] The present invention, in some embodiments thereof, relates to non-graft versus host disease (GVHD) inducing anti-third party cells comprising a central memory T-lymphocyte phenotype and, more particularly, but not exclusively, to the use of same for graft versus leukemia/lymphoma treatment.

[0002] Treatment options for patients with hematological malignancies such as non-Hodgkin lymphoma (NHL) are many and varied. These modem treatment protocols lead to complete remission (CR) in a considerable proportion of the patients. However, many of these patients ultimately relapse, implying that residual tumor cells remain in patients achieving a clinical CR. To address this challenge donor lymphocyte infusion (DLI) endowed with graft-versus-leukemia/lymphoma (GVL) reactivity are currently being developed. In particular, progress has been made in the context of allogeneic bone marrow transplantation (BMT) in conjunction with DLI after transplantation [Grigg A and Ritchie D, Biol Blood Marrow Transplant (2004) 10: 579-590]. Thus, it has been demonstrated that donor CD8+ T cells present in the stem cell graft or in DLI have the added benefit of GVL effect that can kill residual malignant cells [Ho WY et al., J Clin Invest. (2002) 110: 1415-1417]. However, this benefit is offset by graft-versus-host disease (GVHD), associated with CD8 T cells, which adversely impact transplant-related mortality.

[0003] Previous work done by the present inventors has shown that ex-vivo stimulation of murine CD8+ T cells against 3rd-party stimulators, under IL-2 deprivation, leads to selective growth of 3rd-party restricted CD8+ cytotoxic T lymphocyte (CTL) clones which can facilitate T cell depleted BMT (TDBMT) engraftment without causing GVHD [Bachar-Lustig E. et al., Blood (2003) 102:1943-1950; Reich-Zeliger S. et al., Immunity (2000) 13: 507-515]. Recently, the present inventors demonstrated that activated anti-3rd party CD8+ cells with central memory phenotype (Tcm), can further support and improve bone marrow (BM) engraftment, likely due to the improved lymph node homing of the Tcm cells, their proliferative capacity and prolonged persistence in BMT recipients [Ophir E et al., Blood (2010) 115: 2095-2104].

[0004] Furthermore, the present inventors have shown that in humans, anti-3rd party CTLs, although depleted of alloreactivity, exhibit potent in-vitro killing of B-CLL and different types of primary lymphoma cells [Lask A et al. (submitted 2010); Arditti FD et al., Blood (2005) 105:3365-3371]. This unique form of GVL was shown to be independent of TCR recognition and it was found to be mediated both by autologous and by allogeneic anti-3rd party CTLs. Furthermore, this TCR independent killing of B cell malignancies by anti-3rd party CTLs was shown to be mediated via a rapid adhesion through ICAM1-LFA1 binding, followed by slow induction of apoptosis upon a critical interaction between CD8 on the CTL and MHC class I on the tumor cell. Moreover, the killing was shown to be independent of the classical CTLs death molecules: FASL, perforin, TNF, and Trail [Lask A et al., supra].

[0005] Additional background art includes WO/2010/049935, WO 2007/023491 and WO 01/49243.

[0006] Kawai Tatsuo et al relates to HLA-mismatched renal transplantation without maintenance immunosuppression, new England Journal of medicine, massachussets Medical society, Boston, MA, US, vol. 358, No4, 24 January 2008, pages 353-361.

[0007] Ophir E et al relates to induction of tolerance in organ recipients by hematopoietic stem cell transplantation, international Immunopharmacology, Elsevier, Amsterdam, NL, Vol.9, No 6, June 1, 2009, pages 694-700.

SUMMARY OF THE INVENTION

[0008] The present invention relates to a method of generating an isolated population of cells comprising non-graft versus host (GVHD) inducing anti-third party cells having a central memory T-lymphocyte (Tcm) phenotype, said central memory T-lymphocyte (Tcm) phenotype comprising a $CD8^+/CD62L^+/CD45RO+$ signature and wherein at least 50 % of the isolated population of cells have said signature, said cells being tolerance-inducing cells and capable of homing to the lymph nodes following transplantation, the method comprising:

(a) contacting peripheral blood mononuclear cells (PBMC) with a third party antigen or antigens in the presence of IL-21 under conditions which allow elimination of GVH reactive cells; and
(b) culturing said cells resulting from step (a) in the presence of IL-15 in an antigen free environment under conditions which allow proliferation of cells comprising said central memory T-lymphocyte (Tcm) phenotype, thereby generating the isolated population of cells.

[0009] The present invention relates also to an isolated population of cells comprising non-graft versus host (GVHD) inducing anti-third party cells having a central memory T-lymphocyte (Tcm) phenotype, said central memory T-lymphocyte (Tcm) phenotype comprising a $CD8^+/CD62L^+/CD45RO+$ signature and wherein at least 50 % of the isolated population of cells have said signature, said cells being tolerance-inducing cells and capable of homing to the lymph nodes following transplantation, for use as an adjuvant treatment for eradication of a disease in a subject who has been transplanted with a non-syngeneic cell or tissue graft, wherein said cells are either:

(i) non-syngeneic with both the subject and said graft; or
(ii) non-syngeneic with said graft and syngeneic with the subject.

[0010] Preferably said cell or tissue graft is non-autologous and said isolated population of cells are autologous.

[0011] Preferably said cell or tissue graft and said isolated population of cells are from different donors.

[0012] Preferably said disease comprises a malignancy and optionally a B cell malignancy.

[0013] Preferably said graft comprises bone marrow cells and optionally immature hematopoietic cells.

[0014] Preferably when said immature hematopoietic cells are non-syngeneic with the subject, said isolated population of cells are syngeneic with the subject.

[0015] Preferably said immature hematopoietic cells are non-autologous and said isolated population of cells are autologous.

[0016] Preferably when said immature hematopoietic cells are non-syngeneic with the subject, said isolated population of cells are non-syngeneic with both the subject and with said graft.

[0017] Preferably said immature hematopoietic cells and said isolated population of cells are from different donors.

[0018] Preferably said lymph nodes comprise peripheral lymph nodes or mesenteric lymph nodes.

[0019] Preferably said cells non-syngeneic with said graft and syngeneic with the subject comprise autologous cells.

[0020] The present invention relates also to a therapeutically effective amount of an isolated population of cells comprising non-graft versus host (GVHD) inducing anti-third party cells having a central memory T-lymphocyte (Tcm) phenotype, said central memory T-lymphocyte (Tcm) phenotype comprising a $CD8^+/CD62L^+/CD45RO+$ signature and wherein at least 50 % of the isolated population of cells have said signature, said cells being tolerance-inducing cells and capable of homing to the lymph nodes following transplantation for use as an adjuvant treatment for eradication of a disease in a subject who has been transplanted with immature hematopoietic cells, and further wherein when said immature hematopoietic cells are syngeneic with the subject, said isolated population of cells are selected syngeneic with the subject or non-syngeneic with the subject.

[0021] Preferably said immature hematopoietic cells and said isolated population of cells are autologous or wherein said immature hematopoietic cells are autologous and said isolated population of cells are non-autologous.

[0022] Preferably said anti-third party cells having a central memory T-lymphocyte (Tcm) phenotype, said cells being tolerance-inducing cells and capable of homing to the lymph nodes following transplantation are generated by:

(a) contacting peripheral blood mononuclear cells (PBMC) with a third party antigen or antigens in the presence of IL-21 under conditions which allow elimination of GVH reactive cells; and

(b) culturing said cells resulting from step (a) in the presence of IL-15 in an antigen free environment under conditions which allow proliferation of cells comprising said central memory T-lymphocyte (Tcm) phenotype, thereby generating the isolated population of cells.

[0023] Preferably said conditions which allow elimination of GVH reactive cells comprise culturing for 1-5 days.

[0024] Preferably said culturing in the presence of IL-15 is effected for 3-30 days.

[0025] Preferably said conditions which allow proliferation of cells comprising said central memory T-lymphocyte (Tcm) phenotype further comprise IL-7 and/or IL-21. Preferably said disease comprises leukemia or lymphoma.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] 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.

[0027] In the drawings:

FIGs. 1A-I depict inhibition of tumor relapse by syngeneic derived anti-3rd party Tcm cells after syngeneic bone marrow transplantation. Lethally irradiated (8 Gy) BALB/c mice ($H\text{-}2^d$) received intravenously a transplant of 3×10^6 syngeneic T cell depleted bone marrow (BALB/c-NUDE ($H\text{-}2^d$) in the presence or absence of 5×10^3 A20-luc lymphoma cells (day 0). Mice were then intravenously injected with or without the indicated numbers of BALB/c derived anti-3rd party Tcm cells (indicated as leukemia cells + Tcm, n = 7; or with only leukemia cells, n =7, respectively) on day +1. Figures 1A-H are pictures depicting tumor growth monitored by bioluminescence imaging (BLI) from day 14 in weekly intervals; Figure 1I is a graph depicting the survival rate of the animals from the different treatment groups. X-axis indicates days after tumor cell injection and the y-axis indicates the proportion of recipient mice surviving.

FIGs. 2A-G depict inhibition of tumor relapse by F1 derived anti-3rd party CD8 T cells after syngeneic bone marrow transplantation. Lethally irradiated (8 Gy) BALB/c mice ($H\text{-}2^d$) received intravenously a transplant of 3×10^6 syngeneic T cell depleted bone marrow cells (BALB/c-NUDE ($H\text{-}2^d$) in the presence or absence of 5×10^3 A20-luc lymphoma cells (day 0). Mice were then intravenously injected with or

without 2 x 10^7 F1 derived anti-3rd party Tcm cells (Leukemia cells + Tcm, n = 7) or without (Leukemia cells alone, n=5) on day +1; Figures 2A-F are pictures depicting tumor growth monitored by BLI from day 14 in weekly intervals. Figure 2G is a graph depicting the survival rate of the animals from the different treatment groups. X-axis indicates days after tumor cell injection and the y-axis indicates the proportion of recipient mice surviving.

FIGs. 3A-I depict inhibition of tumor relapse by allogeneic derived anti-3rd party Tcm cells after allogeneic bone marrow transplantation. Lethally irradiated (8 Gy) BALB/c (H-2^d) mice received intravenously a transplant of 3 x 10^6 allogeneic T cell depleted bone marrow cells (C57BL/6-NUDE (H-2^b) in the presence or absence of 5 x 10^3 A20-luc lymphoma cells (day 0). Mice were then intravenously injected with C57BL/6 derived cells (on day +1). Figures 3A-H are pictures depicting tumor growth monitored by BLI from day 13 in weekly intervals; Figure 3I is a graph depicting the survival rate of the animals from the different treatment groups. X-axis indicates days after tumor cell injection and the y-axis indicates the proportion of recipient mice surviving.

FIGs. 4A-B are graphs depicting the proliferation and cell phenotype of anti-third party T central memory cells of the present invention. Figure 4A depicts Tcm cell proliferation from day 0 until day 12 of culture; and Figure 4B depicts cell phenotype using the same culture against Allo-DC.

FIG. 5 is a graph depicting the percent of apoptotic cells after 22 hours of mixed lymphocyte reaction (MLR) with B-cell lymphoma and plasma cell leukemia cell lines. CalceinAM pre-labeled Daudi, H.My2 C1R HLA A2 K66A mutant or L363 cell lines were incubated for 22 hours with or without 5-fold excess of anti-3rd party Tcm. Annexin V+ cells were determined by FACS. Data is shown as mean±SD of pentaplicate cultures. ***p<0.001 values indicate statistically significant changes compared to samples cultured in the absence of Tcm.

FIG. 6 is a graph depicting the number of live cells after 22 hours of mixed lymphocyte reaction (MLR) with B-cell lymphoma EBV-LCL and plasma cell leukemia cell lines. CalceinAM pre-labeled Daudi, H.My2 C1R HLA A2 K66A mutant or L363 cell lines were incubated for 22 hours with or without 5-fold excess of anti-3rd party Tcm. Numbers of viable CalceinAM$^+$ cells were determined by FACS. Data is shown as mean±SD of pentaplicate cultures. ***p<0.001 values indicate statistically significant changes compared to samples cultured in the absence of Tcm.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

[0028] The present disclosure, in some embodiments thereof, relates to non-graft versus host disease (GVHD) inducing anti-third party cells comprising a central memory T-lymphocyte phenotype and, more particularly, but not exclusively, to the use of same for graft versus leukemia/lymphoma treatment.

[0029] The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

[0030] 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.

[0031] While reducing the present invention to practice, the present inventors have uncovered that anti-3rd party CD8+ T central memory (Tcm) cells comprise *in vivo* graft versus leukemia/lymphoma (GVL) activity and may therefore be used to treat malignant hematopoietic diseases, such as lymphoma and leukemia.

[0032] As is shown herein below and in the Examples section which follows, the present inventors have shown GVL reactivity of anti-3rd party CD8+ Tcm cells in an *in-vivo* mouse model specifically designed to simulate allogeneic bone marrow transplant (BMT) in lymphoma patients. Initially, inventors confirmed *in-vitro* that non-alloreactive derived murine anti-3rd party Tcm cells act similarly to human anti-3rd party CTLs and directly eradicate A20 murine lymphoma cells (see Example 1 of the Examples section which follows). Subsequently, the present inventors established a minimal residual disease mouse model for B cell lymphoma utilizing A20 B cell lymphoma cell line in which luciferase reporter gene was stably integrated into its genome (A20-luc). These cells enabled sensitive monitoring of *in-vivo* tumor progression by bioluminescence imaging (BLI). Using this model, inventors discovered that both syngeneic and allogeneic anti-3rd party Tcm cells exhibit marked GVL reactivity without causing graft versus host disease (GVHD) when administered in conjunction with a syngeneic bone marrow transplant (Examples 2 and 3, respectively, of the Examples section which follows). Furthermore, the present inventors showed an effective GVL effect devoid of GVHD when administering allogeneic anti-3rd party Tcm cells (donor type) in conjunction with an allogeneic bone marrow transplant (Example 4, of the Examples section which follows). Taken together, all these findings substantiate the use of anti-third party Tcm cells as graft versus leukemia/lymphoma cells for eradication of diseased cells. Moreover, the present results demonstrate the ability to use anti-third party Tcm cells from a donor non-syngeneic with respect to the recipient and to the transplant donor (e.g. from two different donors).

[0033] Thus, according to one aspect of the present disclosure there is provided a method of treating a dis-

ease in a subject in need thereof, the method comprising: (a) transplanting a cell or tissue graft to the subject; and (b) administering to the subject a therapeutically effective amount of an isolated population of cells comprising non-graft versus host (GVHD) inducing anti-third party cells having a central memory T-lymphocyte (Tcm) phenotype, the cells being tolerance-inducing cells and capable of homing to the lymph nodes following transplantation, and further wherein the cells are either: (i) non-syngeneic with both the subject and the graft; or (ii) non-syngeneic with the graft and syngeneic with the subject, thereby treating the subject.

[0034] 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.

[0035] 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 graft. Typically the subject is in need of cell or tissue graft (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 transplantation of a cell or tissue graft. Examples of such disorders are provided further below.

[0036] 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, 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, hematopoietic stem cells (e.g. immature hematopoietic cells).

[0037] According to a specific embodiment, the hematopoietic stem cells of the present disclosureare CD34+.

[0038] Depending on the application, the method may be effected using a cell or tissue graft which is syngeneic or non-syngeneic with the subject.

[0039] As used herein, the term "syngeneic" refers to a cell or tissue which is derived from an individual who is essentially genetically identical with the subject. Typically, essentially fully inbred mammals, mammalian clones, or homozygotic twin mammals are syngeneic.

[0040] Examples of syngeneic cells or tissues include cells or tissues derived from the subject (also referred to in the art as "autologous"), a clone of the subject, or a homozygotic twin of the subject.

[0041] As used herein, the term "non-syngeneic" refers to a cell or tissue which is derived from an individual who is allogeneic or xenogeneic with the subject's lymphocytes (also referred to in the art as "non-autologous").

[0042] 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 with respect to the subject.

[0043] 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.

[0044] The present disclosureenvisages that xenogeneic cells or tissues are derived from a variety of species such as, but not limited to, bovines (e.g., cow), equids (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).

[0045] 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.

[0046] According to an embodiment of the present disclosure, both the subject and the donor are humans.

[0047] Depending on the application and available sources, the cells or tissue grafts of the present disclosuremay be obtained from a prenatal organism, postnatal organism, an adult or a cadaver donor. Moreover, depending on the application needed, the cells or tissues may be naive or genetically modified. Such determinations are well within the ability of one of ordinary skill in the art

[0048] Any method known in the art may be employed to obtain a cell or tissue (e.g. for transplantation).

[0049] Transplanting the cell or tissue graft 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.

[0050] Transplanting a cell or tissue graft of the present disclosuremay 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 bones, the heart cavity, the heart, the

chest cavity, the lung, the skin, the pancreas and/or the intra abdominal space.

[0051] For example, in cases requiring immature hematopoietic cell transplantation, immature autologous, allogeneic or xenogeneic hematopoietic cells (e.g. stem cells) which can be derived, for example, from bone marrow, mobilized peripheral blood (by for example leukapheresis), fetal liver, yolk sac and/or cord blood of the syngeneic or non-syngeneic donor can be transplanted to a recipient suffering from a disease.

[0052] According to an embodiment of the present disclosure, the disease is a malignant disease. According to a specific embodiment, the malignant disease is a malignancy of hematopoietic or lymphoid tissues. According to another specific embodiment, the malignant disease is a B cell malignancy (i.e. involving B lymphocytes).

[0053] Such a disease includes, but is not limited to, leukemia [e.g., acute lymphatic, acute lymphoblastic, acute lymphoblastic pre-B cell, acute lymphoblastic T cell leukemia, acute - megakaryoblastic, monocytic, acute myelogenous, acute myeloid, acute myeloid with eosinophilia, B cell, basophilic, chronic myeloid, chronic, B cell, eosinophilic, Friend, granulocytic or myelocytic, hairy cell, lymphocytic, megakaryoblastic, monocytic, monocytic-macrophage, myeloblastic, myeloid, myelomonocytic, plasma cell, pre-B cell, promyelocytic, subacute, T cell, lymphoid neoplasm, predisposition to myeloid malignancy, acute nonlymphocytic leukemia, T-cell acute lymphocytic leukemia (T-ALL) and B-cell chronic lymphocytic leukemia (B-CLL)], lymphoma [e.g., Hodgkin's lymphoma, non-Hodgkin's lymphoma, B cell, diffuse large B-cell lymphoma (DLBCL), B-cell chronic lymphocytic leukemia/lymphoma, Burkitt's lymphoma, T cell, cutaneous T cell, precursor T-cell leukemia/lymphoma, follicular lymphoma, mantle cell lymphoma, MALT lymphoma, histiocytic, lymphoblastic, thymic and Mycosis fungoides], diseases associated with transplantation of a graft (e.g. graft rejection, chronic graft rejection, subacute graft rejection, hyper-acute graft rejection, acute graft rejection and graft versus host disease), autoimmune diseases such as Type 1 diabetes, severe combined immunodeficiency syndromes (SCID), including adenosine deaminase (ADA), osteopetrosis, aplastic anemia, Gaucher's disease, thalassemia and other congenital or genetically-determined hematopoietic abnormalities.

[0054] It will be appreciated that the syngeneic or non-syngeneic hematopoietic cells (e.g. immature hematopoietic cells) of the present disclosuremay 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.) or via an intraperitoneal route.

[0055] Optionally, when transplanting a cell or tissue graft of the present disclosureinto 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 graft, and structural/functional integration thereof with the anatomy/physiology of the subject.

[0056] Following transplantation of the cell or tissue graft into the subject according to the present teachings, 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, structural development of the cells or tissues may be monitored via computerized tomography or ultrasound imaging while engraftment of non-syngeneic cell or bone marrow grafts can be monitored for example by chimerism testing [e.g. by PCR-based procedures using short tandem repeat (STR) analysis].

[0057] Regardless of the transplant type, to avoid graft rejection and graft versus host disease and to abolish any residual tumor cells, the method of the present disclosureutilizes anti-third party Tcm cells.

[0058] Thus, according to an aspect of the present disclosure, the subject is administered a therapeutically effective amount of an isolated population of cells comprising non-graft versus host (GVHD) inducing anti-third party cells having a central memory T-lymphocyte (Tcm) phenotype, the cells being tolerance-inducing cells and capable of homing to the lymph nodes following transplantation.

[0059] The phrase "isolated population of cells" as used herein refers to cells which have been isolated from their natural environment (e.g., the human body).

[0060] The term "non-GVHD" as used herein refers to having substantially no graft versus host inducing reactivity. Thus, the cells of the present disclosureare generated as to not significantly cause graft versus host disease (GVHD).

[0061] The phrase "anti-third party cells" as used herein refers to lymphocytes (e.g. T lymphocytes) which are directed (e.g. by T cell recognition) against a third party antigen or antigens.

[0062] As used herein the phrase "third party antigen or antigens" refers to a soluble or non-soluble (such as membrane associated) antigen or antigens which are not present in either the donor or recipient, as depicted in detail infra.

[0063] According to one embodiment, the third party antigen or antigens of the present disclosureis selected from the group consisting of third party cells, a cell antigen, a viral antigen, a bacterial antigen, a protein extract, a purified protein and a synthetic peptide presented by autologous presenting cells, non-autologous presenting cells or on an artificial vehicle or artificial antigen presenting cell.

[0064] For example, third party antigens can be third party cells, antigens of viruses, such as for example, Epstein-Barr virus (EBV) or cyto-megalo virus (CMV) or antigens of bacteria, such as flagellin. Viral or bacterial antigens can be presented by cells (e.g., cell line) infected therewith or otherwise made to express viral/bacterial proteins. Autologous or non-autologous antigen presenting cells can be used to present short synthetic peptides

fused or loaded thereto. Such short peptides may be viral derived peptides or peptides representing any other antigen.

[0065] Dedicated software can be used to analyze viral or other sequences to identify immunogenic short peptides, i.e., peptides presentable in context of class I MHC or class II MHC.

[0066] Third party cells can be either allogeneic or xenogeneic with respects to the recipient (explained in further detail below). In the case of allogeneic third party cells, such cells have HLA antigens different from that of the donor but which are not cross reactive with the recipient HLA antigens, such that anti-third party cells generated against such cells are not reactive against a transplant or recipient antigens.

[0067] According to an embodiment of the present disclosure the allogeneic or xenogeneic third party cells are stimulatory cells such as, but not limited to, cells purified from peripheral blood lymphocytes (PBL), spleen or lymph nodes, cytokine-mobilized PBLs, in vitro expanded antigen-presenting dendritic cells (APC), B cell lines, Antigen presenting cells (APC) such as artificial APC (e.g. K562 cell line transfected with HLA and/or costimulatory molecules).

[0068] According to an embodiment, the third party antigen or antigens comprise dendritic cells.

[0069] Third party antigens can be presented on the cellular, viral or bacterial surfaces or derived and/or purified therefrom. Additionally, a viral , bacterial or any foreign antigen can be displayed on an infected cell or can be displayed on an artificial vehicle such as a liposome or an artificial APC (e.g.fibroblast or leukemic cell line transfected with the third party antigen or antigens).

[0070] In addition, third party antigens can, for example, be proteins extracted or purified from a variety of sources. An example of a purified protein which can serve as a third party antigen according to the present disclosureis ovalbumin. Other examples are envisaged.

[0071] Utilizing cells, viruses, bacteria, virally infected, bacteria infected, viral peptides or bacteria peptides presenting cells as third party antigens is particularly advantageous since such third party antigens include a diverse array of antigenic determinants and as such direct the formation of anti-third party cells of a diverse population, which may further serve in faster reconstitution of T-cells in cases where such reconstitution is required, e.g., following lethal or sublethal irradiation or chemotherapy procedure.

[0072] Furthermore, when anti-third party cells are directed against third party antigens, it is of advantage to obtain at least some graft versus disease (e.g. cancer cell such as graft versus leukemia) activity due to TCR independent killing mediated by LFA1-ICAM1 binding [Arditti et al., Blood (2005) 105(8):3365-71. Epub 2004 Jul 6].

[0073] According to some embodiments, the anti-third party cells of the present disclosurecomprise a central memory T-lymphocyte (Tcm) phenotype.

[0074] The phrase "central memory T-lymphocyte (Tcm) phenotype" as used herein refers to a subset of T cytotoxic cells which home to the lymph nodes. Cells having the Tcm phenotype, in humans, typically express CD8+/CD62L+/CD45RO+/L-selectin+/CD45RA-. According to a more specific embodiment the Tcm phenotype comprises a CD8+/CD62L+ signature. It will be appreciated that Tcm cells may express all of the signature markers on a single cell or may express only part of the signature markers on a single cell.

[0075] It will be appreciated that at least at least 30 %, at least 40 %, at least 50 %, at least 55 %, at least 60 %, at least 65 %, at least 70 %, at least 75 %, at least 80 %, at least 85 %, at least 90 %, at least 95 % or at least 100 % of the isolated population of cells comprise cells having the Tcm cell signature.

[0076] As mentioned, the Tcm cells typically home to the lymph nodes following transplantation. According to some embodiments the anti-third party Tcm cells of the present disclosuremay home to any of the lymph nodes following transplantation, as for example, the peripheral lymph nodes and mesenteric lymph nodes. The homing nature of these cells allows them to exert their tolerance effect in a rapid and efficient manner.

[0077] Thus, the anti-third party Tcm cells of the present disclosureare tolerance-inducing cells.

[0078] The phrase "tolerance inducing cells" as used herein refers to cells which provoke decreased responsiveness of the recipient's cells (e.g. recipient's T cells) when they come in contact with same. Tolerance inducing cells include veto cells (i.e. T cells which lead to apoptosis of host T cells upon contact with same) as was previously described in PCT Publication Nos. WO 2001/049243 and WO 2002/102971.

[0079] The use of tolerance inducing cells is especially beneficial in situations in which there is a need to eliminate graft rejection and overcome graft versus host disease (GVHD), such as in transplantation of allogeneic or xenogeneic cells or tissues.

[0080] According to some embodiments, the Tcm cells of the present disclosuremay be naïve cells (e.g. non-genetically modified) or genetically modified cells (e.g. cells which have been genetically engineered to express or not express specific genes, markers or peptides or to secrete or not secrete specific cytokines). Any method known in the art may be implemented in genetically engineering the cells, such as by inactivation of the relevant gene/s or by insertion of an antisense RNA interfering with polypeptide expression (see e.g. WO/2000/039294).

[0081] Any method used for the generation of anti-third party non-alloreactive cells (devoid of graft versus host (GVH) activity) can be used in accordance with the present teachings.

[0082] The anti-third party Tcm cells of the present disclosureare typically generated by first contacting syngeneic or non-syngeneic peripheral blood mononuclear cells (PBMC) with a third party antigen or antigens (such

as described above) in a culture deprived of cytokines (i.e.,. without the addition of cytokines). This step is typically carried out for about 12-72 hours, 24-48 hours, 1-10 days, 1-7 days, 1-5 days, 2-3 days or 2 days and allows elimination of GVH reactive cells (e.g. T cells). Alternatively, the anti-third party Tcm cells may be generated devoid of graft versus host (GVH) activity by supplementing the otherwise cytokine-free culture with IL-21 (0.001-3000 ng/ml, 10 - 1000 ng/ml, 10-100 ng/ml, 1-100 ng/ml, 0.1-100 ng/ml, 0.1-10 ng/ml, 1-50 ng/ml or 1-10 ng/ml). This step is typically carried out for about 12-72 hours, 24-48 hours, 1-10 days, 1-10 days, 1-7 days, 1-5 days, 2-3 days or 3 days.

[0083] Next, the anti-third party cells are cultured in the presence of IL-15 (0.05-500 ng/ml, 0.001-3000 ng/ml, 10 - 1000 ng/ml, 10-100 ng/ml, 1-100 ng/ml, 0.1-100 ng/ml, 0.1-10 ng/ml, 1-50 ng/ml or 1-10 ng/ml. According to a specific embodiment the concentration is 5 ng/ml) for a period of about 3-30 days, 6-30 days, 3-20 days, 10-20 days, 3-15 days, 5-15 days, 7-15 days, 7-14 days, 3-10 days, 3-7 days or 14 days in an antigen-free environment. The culture may be further effected in the presence of additional cytokines such as IL-7 (0.05-500 ng/ml, 0.001-3000 ng/ml, 10 - 1000 ng/ml, 10-100 ng/ml, 1-100 ng/ml, 0.1-100 ng/ml, 0.1-10 ng/ml, 1-50 ng/ml or 1-10 ng/ml. According to a specific embodiment the concentration is 5 ng/ml) and/or IL-21 (0.001-3000 ng/ml, 0.001-3000 ng/ml, 10 - 1000 ng/ml, 10-100 ng/ml, 1-100 ng/ml, 0.1-100 ng/ml, 0.1-10 ng/ml, 1-50 ng/ml or 20-50 ng/ml. According to a specific embodiment the concentration is 30 ng/ml). This process enables proliferation of anti-third party cells comprising a central memory T-lymphocyte (Tcm) phenotype and being deprived of GVHD reactivity.

[0084] It will be appreciated that an additional step which allows selection of $CD8^+$ T cells may be carried out, such as by the use of MACS beads, before culturing the cells in the presence of IL-15. Such a step may be beneficial in order to increase the purity of the $CD8^+$ cells within the culture (i.e. eliminate other lymphocytes within the cell culture e.g. T $CD4^+$ cells) or in order to increase the number of $CD8^+$ T cells. Thus, isolation of CD8+ cells can be done prior to culturing with the third party antigen or antigens or following culturing with the third party antigen or antigens and prior to culturing with CD15.

[0085] According to some embodiments of the disclosure, syngeneic PBMCs (e.g. from the subject) may be used according to the present teachings (i.e. in situations when syngeneic Tcm cells may be beneficial for treatment). Likewise, non-syngeneic PBMCs (e.g. allogeneic or xenogeneic with respect to the subject) may be used according to the present teachings. The source of the PBMCs will be determined with respect to the intended use of the cells (see further details hereinbelow) and is well within the capability of one skilled in the art, especially in light of the detailed disclosure provided herein.

[0086] As described in detail in the Examples section which follows, the present inventors have shown that the anti-third party Tcm cells may be of the same origin as the cell or tissue graft (e.g. bone marrow cells), specifically, they may both be derived from a syngeneic donor (e.g. from the subject, see Example 2 and Figures 1A-I) or may both be derived from a non-syngeneic donor (e.g. from an allogeneic donor, see Example 4 and Figures 3A-I). Conversely, the anti-third party Tcm cells may be from a different origin compared to the cell or tissue graft (e.g. the bone marrow cells may be from the subject and the anti-third party cells may be from an allogeneic donor, Example 3 and Figures 2A-G).

[0087] Thus, according to an embodiment of the present disclosure, the anti-third party Tcm cells may be non-syngeneic (e.g. allogeneic or xenogeneic) with both the subject and the graft.

[0088] As used herein, the phrase "non-syngeneic with both the subject and the graft" when relating to anti-third party Tcm cells of the present disclosurequalifies the anti-third party Tcm cells as being allogeneic or xenogeneic with the subject, and allogeneic or xenogeneic with the graft in any combination. Thus, the anti-third party Tcm cells may be obtained from an origin different from the subject and from the graft donor.

[0089] According to a specific embodiment, the anti-third party Tcm cells are non-syngeneic with both the subject and the graft (e.g. from a second donor).

[0090] According to another embodiment, the anti-third party Tcm cells may be non-syngeneic with respect to only the subject. According to another embodiment, the anti-third party Tcm cells may be non-syngeneic with respect to only the cell or tissue graft.

[0091] According to one embodiment, the anti-third party Tcm cells are non-syngeneic with the graft and syngeneic with the subject (e.g. of an autologous origin in situations in which the graft is of a non-autologous origin).

[0092] According to a specific embodiment, when the graft comprises immature hematopoietic cells which are non-syngeneic with the subject (e.g. non-autologous), the isolated population of cells are syngeneic with the subject (e.g. autologous).

[0093] As used herein, the term "immature hematopoietic cells" refers to any type of incompletely differentiated cells which are capable of differentiating into one or more types of fully differentiated hematopoietic cells. Immature hematopoietic cells include without limitation types of cells referred to in the art as "progenitor cells", "precursor cells", "stem cells", "pluripotent cells", "multipotent cells", and the like.

[0094] Preferably the immature hematopoietic cells are hematopoietic stem cells.

[0095] Preferably, where the immature hematopoietic cells are derived from a human, the immature hematopoietic cells are CD34+ cells, such as CD34+CD133+ cells.

[0096] Types of grafts of the present disclosurewhich comprise immature hematopoietic cells include whole bone marrow cell grafts (T-cell depleted or non-T-cell-depleted), grafts of immature hematopoietic cells from

bone marrow aspirates, grafts of peripheral blood-derived immature hematopoietic cells and grafts of umbilical cord-derived immature hematopoietic cells. Methods of obtaining such grafts are described hereinbelow.

[0097] A graft which comprises human peripheral blood-derived hematopoietic stem cells may be obtained according to standard methods, for example by mobilizing CD34+ cells into the peripheral blood by cytokine treatment of the donor, and harvesting of the mobilized CD34+ cells via leukapheresis. Ample guidance is provided in the literature of the art for practicing isolation of bone marrow-derived stem cells from the bone marrow or the blood (refer, for example, to: Arai S, Klingemann HG., 2003. Arch Med Res. 34:545-53; and Repka T. and Weisdorf D., 1998. Curr Opin Oncol. 10:112-7; Janssen WE. et al., 1994. Cancer Control 1:225-230; Atkinson K., 1999. Curr Top Pathol. 92:107-36).

[0098] A graft of human umbilical cord blood-derived hematopoietic stem cells may be obtained according to standard methods (refer, for example, to: Quillen K, Berkman EM., 1996. J Hematother. 5:153-5).

[0099] A graft of hematopoietic stem cells of the present disclosuremay also be derived from liver tissue or yolk sac.

[0100] A requisite number of hematopoietic stem cells can be provided by *ex-vivo* expansion of primary hematopoietic stem cells (reviewed in Emerson, 1996, Blood 87:3082, and described in more detail in Petzer et al., 1996, Proc. Natl. Acad. Sci. U. S. A. 3:1470; Zundstra et al., 1994, BioTechnology 12:909; and WO 95 11692). According to another specific embodiment, when the graft comprises immature hematopoietic cells which are non-syngeneic with the subject (e.g. non-autologous), the isolated population of cells are non-syngeneic with both the subject and with the graft. (e.g. the immature hematopoietic cells and the isolated population of cells are from different donors).

[0101] According to another embodiment, there is provided a method of treating a disease in a subject in need thereof, the method comprising: (a) transplanting immature hematopoietic cells to the subject; and (b) administering to the subject a therapeutically effective amount of an isolated population of cells comprising non-graft versus host (GVHD) inducing anti-third party cells having a central memory T-lymphocyte (Tcm) phenotype, the cells being tolerance-inducing cells and capable of homing to the lymph nodes following transplantation, wherein when the immature hematopoietic cells are syngeneic with the subject, the isolated population of cells are selected syngeneic with the subject or non-syngeneic with the subject.

[0102] According to a specific embodiment, both the immature hematopoietic cells and the isolated population of cells are autologous (e.g. from the subject).

[0103] According to another specific embodiment, the immature hematopoietic cells are autologous (e.g. from the subject) and the isolated population of cells are non-autologous (e.g. from a donor).

[0104] According to another embodiment, there is provided a method of treating a disease in a subject in need thereof, the method comprising: (a) transplanting immature hematopoietic cells to the subject; and (b) administering to the subject a therapeutically effective amount of an isolated population of cells comprising non-graft versus host (GVHD) inducing anti-third party cells having a central memory T-lymphocyte (Tcm) phenotype, the cells being tolerance-inducing cells and capable of homing to the lymph nodes following transplantation, wherein when the immature hematopoietic cells are non-syngeneic with the subject, the isolated population of cells are selected syngeneic with the subject or non-syngeneic with both the subject and the immature hematopoietic cells.

[0105] According to a specific embodiment, the immature hematopoietic cells are non-autologous (e.g. from a donor) and the isolated population of cells are autologous (e.g. from the subject).

[0106] According to another embodiment, when the immature hematopoietic cells are non-syngeneic with the subject (e.g. non-autologous), the anti-third party Tcm cells are non-syngeneic with both the subject and the graft (e.g. two different donors).

[0107] According to a specific embodiment, the immature hematopoietic cells and the isolated population of cells are from different donors.

[0108] According to an additional aspect of the present disclosure, there is provided an isolated population of cells comprising non-graft versus host (GVHD) inducing anti-third party cells having a central memory T-lymphocyte (Tcm) phenotype, the cells being tolerance-inducing cells and capable of homing to the lymph nodes following transplantation, and further wherein the cells are non-syngeneic with both a subject and a cell or tissue graft.

(i) non-syngeneic with both a host subject and a graft; or

(ii) non-syngeneic with a graft and syngeneic with a host subject.

[0109] According to one embodiment, the cells non-syngeneic with the graft and syngeneic with the host subject are autologous.

[0110] Thus, the present disclosurecontemplates administration to a subject any anti-third party Tcm cells (e.g. non-syngeneic with both the subject and the graft or non-syngeneic with the graft and syngeneic with the subject) which will result in eradication of a disease (e.g. leukemia or lymphoma) and will concomitantly enhance engraftment of a cell or tissue transplant (e.g. autologous or non-autologous bone marrow cells) by being tolerogenic cells and non-GVHD.

[0111] It will be appreciated that the anti-third party cells may be administered concomitantly with a cell or tissue graft (e.g. as an adjuvant therapy), may be administered prior to transplantation of a cell or tissue graft (e.g.

in order to eradicate residual cancer cells prior to transplantation and to eliminate graft rejection and graft versus host disease), or may be administered following transplantation of a cell or tissue graft (e.g. in order to eradicate residual cancer cells following transplantation and to eliminate graft rejection and graft versus host disease).

[0112] It will be appreciated that the anti-third party cells may be administered at any time following transplantation. Typically, the anti-third party Tcm cells are administered on day 0, day 1, day 2, day 3, day 4, day 5, day 6, day 7, day 8 or day 10 following transplantation. However, the anti-third party Tcm cells may be administered extended times after transplantation, as for example, two weeks, a month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 18 months or 24 months following transplantation.

[0113] The anti third party Tcm cells may be administered via any method known in the art for cell transplantation, such as but not limited to, cell infusion (e.g. I.V.) or via an intraperitoneal route.

[0114] Without being bound to theory, a therapeutically effective amount is an amount of anti-third party Tcm cells efficient for tolerization, anti-tumor effect and/or immune reconstitution without inducing GVHD. Since the Tcm cells of the present disclosurehome to the lymph nodes following transplantation, lower amounts of cells (compared to the dose of cells previously used, see for example WO 2001/049243) may be needed to achieve the beneficial effect/s of the cells (e.g. tolerization, anti-tumor effect and/or immune reconstitution). It will be appreciated that lower levels of immunosuppressive drugs may be needed in conjunction with the Tcm cells of the present disclosure (such as exclusion of rapamycin from the therapeutic protocol).

[0115] Determination of the therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0116] For any preparation used in the methods of the disclosure, the therapeutically effective amount or dose can be estimated initially from in vitro and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

[0117] For example, in case of tissue graft the number of anti-third party Tcm cells infused to a recipient should be more than 1 x 10^4 /Kg body weight. The number of anti-third party Tcm cells infused to a recipient should typically be in the range of 1 x 10^4 /Kg body weight to 1 x 10^9 /Kg body weight.

[0118] In order to facilitate engraftment of the cell or tissue graft, the method may further advantageously comprise conditioning the subject with an immunosuppressive regimen prior to, concomitantly with, or following transplantation of the cell or tissue graft.

[0119] Thus, according to an embodiment of the present disclosure, the subject is conditioned under sublethal, lethal or supralethal conditions prior to transplantation of a cell or tissue graft.

[0120] For example, the subject may be treated with a myeloablative or non-myeloablative conditioning. The type of conditioning may be determined by one of ordinary skill in the art and takes into account the age and disease severity of the subject. Thus, for example, an elderly subject (e.g. one who is over 40 years of age) may be treated with a mild immunosuppressive regimen.

[0121] Examples of suitable types of immunosuppressive regimens include administration of immunosuppressive drugs, tolerance inducing cell populations (as described in detail hereinabove), and/or immunosuppressive irradiation.

[0122] 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).

[0123] Preferably, the immunosuppressive regimen consists of administering at least one immunosuppressant agent to the subject.

[0124] Examples of immunosuppressive agents include, but are not limited to, methotrexate, cyclophosphamide, cyclosporine, cyclosporin A, chloroquine, hydroxychloroquine, sulfasalazine (sulphasalazopyrine), gold salts, D-penicillamine, leflunomide, azathioprine, anakinra, infliximab (REMICADE), etanercept, TNF.alpha. blockers, a biological agent that targets an inflammatory cytokine, and Non-Steroidal Anti-Inflammatory Drug (NSAIDs). Examples of NSAIDs include, but are not limited to acetyl salicylic acid, choline magnesium salicylate, diflunisal, magnesium salicylate, salsalate, sodium salicylate, diclofenac, etodolac, fenoprofen, flurbiprofen, indomethacin, ketoprofen, ketorolac, meclofenamate, naproxen, nabumetone, phenylbutazone, piroxicam, sulindac, tolmetin, acetaminophen, ibuprofen, Cox-2 inhibitors, tramadol, rapamycin (sirolimus) and rapamycin analogs (such as CCI-779, RAD001, AP23573). These agents may be administered individually or in combination.

[0125] As used herein the term "about" refers to ± 10 %.

[0126] The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

[0127] The term "consisting of means "including and limited to".

[0128] The term "consisting essentially of" means that the composition, method or structure may include addi-

tional 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.

[0129] 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.

[0130] 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.

[0131] 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.

[0132] 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.

[0133] 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.

[0134] 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

[0135] Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

[0136] 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);. 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.

GENERAL MATERIALS AND EXPERIMENTAL PROCEDURES

Animals

[0137] Female 6 to12 week old BALB/c, CB6 (F1),

FVB, C57BL/6, and BALB/c-NUDE mice were obtained from Harlan Laboratories. Progeny of B6-NUDE mice were bred at the Weizmann Institute Animal Center. All mice were kept in small cages (5 animals in each cage) and fed sterile food and acid water. All studies were approved by the Weizmann Institute of Science Institutional Animal Care and Use Committee.

Cells

[0138] The A20 murine lymphoma cell line, a BALb/c (H-2^d) derived B cell lymphoma/leukemia line, previously described [Kim KJ et al., J. Immunol. (1979) 122: 549-554], and the stable transfectant of A20, the A20 yfp/luc$^+$, previously described [Edinger M et al., Blood. (2003) 101: 640-648] were both maintained in RPMI 1640 medium supplemented with 5 % FCS, 2 mM glutamine, nonessential amino acids, antibiotics, and 50 μM 2-βmercaptoethanol.

Preparation of host nonreactive anti-3rd-party Tcm

[0139] Anti-third-party Tcm were prepared as previously described [Ophir E et al., Blood (2010) 115: 2095-2104] briefly, splenocytes of the donor mice were cultured against irradiated third-party splenocytes for 60 hours under cytokine deprivation. Subsequently, CD8$^+$ cells were positively selected using Magnetic Particles (BD Pharmingen) and cultured in an Ag-free environment. rhIL-15 (20 ng/mL; R&D Systems) was added every second day. To attain a purified population at the end of the culture (day 16), the Tcm cells were positively selected for CD62L expression by magnetic-activated cell sorting [MACS, Milteny, Bergisch Gladbach, Germany].

Flow cytometric analysis

[0140] Fluorescence-activated cell sorting (FACS) analysis was performed using a modified Becton Dickinson FACScan. Cells were stained with labeled antibodies specific for CD8-phycoerythrin (PE)/fluorescein isothiocyanate (FITC)/allophycocyanin (APC) (BD Pharmingen), CalceinAM (Molecular Probes, INC., Eugene, OR, USA). Annexin V and 7-amino-actinomycin D (7AAD) staining were done according to the manufacturer's instructions (BD Pharmingen).

MLR culture and cytotoxicity assay

[0141] Anti-3rd-party Tcm and lymphoma cells were obtained by Ficoll density gradient centrifugation and lymphoma cells were labeled with 0.15 μg/ml CalceinAM (Molecular Probes, INC., Eugene, OR, USA) according to manufacturer's instructions and brought to a concentration of 1 x 10^6 cells/ml in the required media. 3 x 10^5 of the lymphoma cells were incubated with anti-3rd-party CTLs according to the indicated ratios and time intervals for 24-well plates. Cells were recovered and analyzed for survival using surface markers such as Annexin-V (BD) and by measuring the number of CalceinAM stained lymphoma cells by FACS.

Detection of apoptosis by Annexin V staining

[0142] Annexin V APC was used to detect apoptotic cells. Samples from *in-vitro* cultures were incubated with a mixture of selected monoclonal antibodies labeled with different fluorochromes for 20 minutes at 4 °C. After washing off the unbound free antibody using an Annexin-V binding buffer, samples were supplemented with 5 μl AnnexinV-APC (BD). Cells were then incubated at room temperature for 15 minutes in the dark and washed with an Annexin-V binding buffer. Samples were analyzed by FACS for live cells that are positively stained of AnnexinV.

Minimal residual disease *in- vivo* model

[0143] 12 week old BALB/c female recipient mice were exposed to a single dose of 8 Gy total body irradiation (TBI) from a Gamma beam 150-A 60 Co source (manufactured by the Atomic Energy of Canada, Kanata, ON, Canada) (day -1). On the following day (day 0) recipient mice were intravenously infused with 3 x 10^6 T cell depleted bone marrow from BALb/c-Nude mice (syngeneic) or with 3 x 10^6 T cell depleted bone marrow from B6-Nude mice, supplemented with 5 x 10^3 A20 luc lymphoma cells per mouse. On the subsequent day (day +1) mice received or did not receive 10 x 10^6 BALB/c derived anti-3rd-party Tcm (syngeneic) or 5 x 10^6 C57BL/6 derived anti-3rd-party Tcm (allogeneic), intravenously. Tumor localization, migratory patterns of A20 cells and the anti-lymphoma reactivity of anti-3rd-party CTLs was surveyed using an *in-vivo* imaging system.

***In-vivo* imaging.**

[0144] Mice were anaesthized with Ketamine (100 mg/kg intra- peritoneally (i.p) (Kepro Holand Netherlands) and Xylazine (Kepro Holand Netherlands) (20 mg/Kg i.p), and an aqueous solution of D Luciferin (150 mg/Kg i.p) (Cat#XR-1001, 30 mg/ml in PBS; Xenogen) was injected 10 minutes prior to imaging. Animals were placed into the light-tight chamber of the In Vivo Imaging system (IVIS® 100, Xenogen) coupled with a Pixelfly QE (PCO, K, Germany) charge-coupled device (CCD) camera at the Department of Veterinary Resources of the Weizmann Institute. A grayscale body surface reference image (digital photograph) was taken after a 10 second exposure, under strong illumination. Image data processing and analysis were performed using Living Image 2.5 software. The mice were monitored for tumor growth from day 14 on weekly intervals.

Statistical analysis

[0145] The analysis of survival data was performed us-

ing Kaplan-Meier curves (log-rank test). Comparison of means was conducted using the Student t test.

EXAMPLE 1

Establishment of a mouse model

[0146] To establish an appropriate mouse model, inventors initially verified that anti-3rd party Tcm cells derived from (B6 x BALB/c)F1 exhibit TCR independent killing of A20 lymphoma cells of BALB/c origin (34.8 ± 12.1 % in 4 experiments, 5:1 Tcm/lymphoma cell ratio, in comparison to A20 cells incubated without Tcm, $p<0.05$). Moreover, after 16 hours of incubation with Tcm cells, AnnexinV staining of A20 cells was significantly enhanced compared to basal staining level (14.8 ± 4.5 % and 5.2 ± 2.2 % respectively, in 3 experiments, $p<0.05$), suggesting an apoptosis based mechanism, similar to that previously described for killing of human lymphoma cells [Lask A et al. (submitted 2010); Arditti FD et al., Blood (2005) 105:3365-3371].

EXAMPLE 2

Treatment protocol by syngeneic bone marrow transplant and Tcm cells

[0147] Using luciferase expressing A20 cells, inventors were able to follow the fate of the malignant cells *in vivo,* and study the anti-lymphoma effect of added donor anti-3rd party Tcm to either syngeneic or allogeneic bone marrow transplant (BMT, hereinbelow), in a model simulating minimal residual disease. In the syngeneic model, 3 x 10^6 Nude BALB/c BM cells were transplanted into lethally irradiated BALB/c mice together with 5000 A20 cells. On the next day, syngeneic Tcm were infused. As can be seen in Figures 1A-D, none of the untreated mice survived (0/7) 100 days post bone marrow transplant (BMT, 23 days median survival). However, administration of 1 x 10^7 or 2 x 10^7 syngeneic Tcm cells led to significantly diminished tumor burden (Figures 1E-H) and improved overall survival of 28 % of mice (2/7, $P<0.0001$) and 40 % of mice (2/5, $P<0.002$) 100 days post BMT, with median survival of 49 and 80 days, respectively (Figure 1I).

EXAMPLE 3

Treatment protocol by syngeneic bone marrow transplant and allogeneic Tcm cells

[0148] An earlier study performed in our lab [Ophir E. et al., Blood (2010) 115: 2095-2104] showed that anti-3rd party Tcm cells are endowed with tolerizing activity, translated into prolonged persistence following BMT, even when using partially matched donors. Inventors therefore tested in the above described syngeneic BMT model the anti-lymphoma effect of 2 x 10^7 F1 (CB6) derived Tcm cells, replacing the syngeneic Tcm cells administered previously. Although these F1 derived Tcm cells were expected to be rejected due to MHC disparity, they were continuously present and not rejected even 2 month after transplantation (data not shown). Their long term persistence was probably the result of their tolerizing activity described above. The significant clinical effect of these F1 derived Tcm cells was demonstrated by an improved overall survival of 57.1 % mice (4/7, Figures 2D-G) in comparison to 0 % mice (0/5, Figures 2A-C and 2G) survival in the untreated group. Thus, these results conclusively demonstrate that anti-3rd party Tcm cells, derived from allogeneic donors, may be used as anti-B cell malignancy cell therapy, either alone or combined with bone marrow cells.

EXAMPLE 4

Treatment protocol by allogeneic bone marrow transplant and allogeneic Tcm cells

[0149] When examined in the allogeneic settings, further improvement of tumor eradication was exhibited. This effect was probably due to residual alloreactivity which provided an additional graft versus leukemia/lymphoma (GVL) effect over the newly discovered T cell receptor (TCR) independent cell killing. Significantly, this additive effect was achieved without causing GVHD. In this allogeneic model, 3 x 10^6 allogeneic Nude B6 BM cells were transplanted together with 5000 A20 cells into lethally irradiated BALB/c mice. On the following day, mice were treated with donor type Tcm. Similar to the results in the syngeneic model (described in Examples 2 and 3, above), none of the untreated mice survived 100 days post BMT (0/8, 23 days median survival, Figures 3A-D and 3I), while administration of 5 x 10^6 donor type Tcm cells, led to remarkable overall survival of 100 % (7/7) 100 days post BMT (Figures 3E-I). Although the allogeneic Tcm cells displayed enhanced GVL activity compared to syngeneic Tcm cells, this effect was not associated with any manifestation of GVHD. Thus, as previously described, the weight and overall appearance of mice receiving allogeneic anti-3rd party Tcm cells were the same as that of mice in the control group, radio-protected with a transplant of Nude BM alone.

[0150] Collectively, the present inventors demonstrated for the first time, by *in vivo* imaging, the GVL reactivity of murine anti-3rd party Tcm. These results suggest that anti-3rd party Tcm cells can provide a 'double supportive effect' by promoting both BM engraftment, and concurrently inducing GVL reactivity without causing GVHD. Such cell therapy is highly attractive, in particular for elderly patients with B-CLL and other B cell malignancies who might not tolerate aggressive conditioning, and can be potentially developed into an 'off the shelf readily available product, to be used as an anti-cancer cell therapy.

EXAMPLE 5

Generation of anti-third party T central memory cells

MATERIALS AND EXPERIMENTAL PROCEDURES

Enrichment of Naïve CD8+ T cell Cells

[0151] Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll density gradient centrifugation of buffy coats from healthy donors. Donor's PBMCs were then transferred to a 10 % DMSO freezing solution and were cryopreserved in liquid nitrogen.

[0152] On day -2: Donor's Frozen PBMCs were thawed quickly in a 37 °C water bath and transferred to warm thawing medium (Cellgro DC supplemented with 10 % human serum and Pen/Strep medium supplemented with Benzonase® Nuclease to avoid cell clump formation as a result of dying cells). Thawed donor's PBMCs were washed twice with warm thawing medium. In order to deplete adherent cells, donor's PBMCs were then resuspended in culture medium (Cellgro DC supplemented with 5 % human Serum and Pen/Strep and 10 ng/ml IL-7) and were plated on specially coated 6-well plates for overnight incubation at 37 °C.

[0153] On day -1: Non-adherent cells were removed (this process increased the concentration of the desires T cell, by removing the adhered monocytes and in addition allowed the thawed cells to recover from the thawing process before being subjected to the magnetic enrichment process).

[0154] On day 0: Untouched CD8+ T-cells were isolated using the CD8 isolation kit from Miltenyi in accordance with the manufacturer's protocol. Thereafter, CD8 T cells with naive phenotype were obtained from the total CD8 population by depleting cells expressing the activation marker CD45RO using CD45RO magnetic beads.

Generation of Monocyte derived dendritic cells (DCs)

[0155] Monocyte derived DCs were generated from allogeneic cryopreserved PBMC. The monocytes were enriched from the PBMC by plastic adherence and cultured in GM-CSF and IL-4 over the course of 3 days. Maturation was induced over the final 24 hours of culture with the addition of LPS and IFN-γ.

Generation of anti-third party Tcm cells (naïve CD8 T cells targeting monocyte derived DCs)

[0156] Naive CD8+ T cells were activated with irradiated (30 gy) monocyte derived DCs at a ratio of 1:0.25, in culture medium (Cellgro DC supplemented with 5 % humaSerum and Pen/Strep) supplemented with IL-21 for 3 days. Thereafter, the CD8+ T cells received no further activation and were grown with IL-7 and IL-15 until day 12.

RESULTS

[0157] On day 11 of culture, cell composition and cell phenotype was evaluated using FACS analysis and cell number was determined by trypan blue exclusion. The results indicated that the cell composition (from Lymphogate, data not shown) comprised predominantly of 94.6 % CD8+ T cells (CD3+CD8+), with traces of 1.2 % CD56+ NK cells (CD3-CD56+) and 1.1 % NK T cell (CD3+CD56+). Furthermore, the CD8+ T cells comprised largely of 76 % Tcm cells, 9 % Naive cells, 8.5 % Teff/Tem and 6.5 % Temra.

EXAMPLE 6

Tcm graft versus leukemia (GVL) assay

MATERIALS AND EXPERIMENTAL PROCEDURES

GVL assay

[0158] B cell lines were labeled with 0.15 μg/ml CalceinAM, a vital dye that is released upon cell death, according to manufacturer's instructions. Next, 0.3 $x10^6$ Calcein labeled cell lines were incubated with or without $1.5 x10^6$ anti-3rd party Tcm for 22 hours in 24 well plates. No exogenous cytokines were added to the MLR. After 22 hours Cells were recovered and analyzed for survival by measuring the number of surviving Calcein stained cells by FACS.

[0159] To obtain absolute values of cells, samples were suspended in constant volume and flow cytometric counts for each sample were obtained during a constant, predetermined period of time and were compared with flow cytometric counts obtained with fixed volume and fixed numbers of input cells.

Detection of apoptosis

[0160] For Detection of apoptosis by AnnexinV staining samples were incubated with 5 μl AnnexinV-APC (BD) for 10 minutes at room temperature. Subsequently, unbound AnnexinV was washed out, and samples were analyzed by FACS.

Claims

1. An in vitro method of generating an isolated population of cells comprising non-graft versus host (GVHD) inducing anti-third party cells having a central memory T-lymphocyte (Tcm) phenotype, said central memory T-lymphocyte (Tcm) phenotype comprising a $CD8^+/CD62L^+/CD45RO+$ signature and wherein at least 50 % of the isolated population of cells have said signature, said cells being tolerance-inducing cells and capable of homing to the lymph nodes following transplantation, the method

comprising:

(a) contacting peripheral blood mononuclear cells (PBMC) with a third party antigen or antigens in the presence of IL-21 under conditions which allow elimination of GVH reactive cells; and
(b) culturing said cells resulting from step (a) in the presence of IL-15 in an antigen free environment under conditions which allow proliferation of cells comprising said central memory T-lymphocyte (Tcm) phenotype, thereby generating the isolated population of cells.

2. An isolated population of cells comprising non-graft versus host (GVHD) inducing anti-third party cells having a central memory T-lymphocyte (Tcm) phenotype, said central memory T-lymphocyte (Tcm) phenotype comprising a $CD8^+/CD62L^+/CD45RO+$ signature and wherein at least 50 % of the isolated population of cells have said signature, said cells being tolerance-inducing cells and capable of homing to the lymph nodes following transplantation, for use as an adjuvant treatment for eradication of a disease in a subject who has been transplanted with a non-syngeneic cell or tissue graft, wherein said cells are either:

(i) non-syngeneic with both the subject and said graft; or
(ii) non-syngeneic with said graft and syngeneic with the subject.

3. The isolated population of cells for use according to claim 2, wherein said cell or tissue graft is non-autologous and said isolated population of cells are autologous.

4. The isolated population of cells for use according to claim 2, wherein said cell or tissue graft and said isolated population of cells are from different donors.

5. The isolated population of cells for use according to claim 2, wherein said disease comprises a malignancy and optionally a B cell malignancy.

6. The isolated population of cells for use according to claim 2, where said graft comprises bone marrow cells and optionally immature hematopoietic cells.

7. The isolated population of cells for use according to claim 6, wherein when said immature hematopoietic cells are non-syngeneic with the subject, said isolated population of cells are syngeneic with the subject.

8. The isolated population of cells for use according to claim 7, wherein said immature hematopoietic cells are non-autologous and said isolated population of cells are autologous.

9. The isolated population of cells for use according to claim 6, wherein when said immature hematopoietic cells are non-syngeneic with the subject, said isolated population of cells are non-syngeneic with both the subject and with said graft.

10. The isolated population of cells for use according to claim 9, wherein said immature hematopoietic cells and said isolated population of cells are from different donors.

11. The method of claim 1, or isolated population of cells for use according to claim 2, wherein said lymph nodes comprise peripheral lymph nodes or mesenteric lymph nodes.

12. The isolated population of cells for use according to claim 2, where said cells non-syngeneic with said graft and syngeneic with the subject comprise autologous cells.

13. A therapeutically effective amount of an isolated population of cells comprising non-graft versus host (GVHD) inducing anti-third party cells having a central memory T-lymphocyte (Tcm) phenotype, said central memory T-lymphocyte (Tcm) phenotype comprising a $CD8^+/CD62L^+/CD45RO+$ signature and wherein at least 50 % of the isolated population of cells have said signature, said cells being tolerance-inducing cells and capable of homing to the lymph nodes following transplantation for use as an adjuvant treatment for eradication of a disease in a subject who has been transplanted with immature hematopoietic cells, and further wherein when said immature hematopoietic cells are syngeneic with the subject, said isolated population of cells are selected syngeneic with the subject or non-syngeneic with the subject.

14. The isolated population of cells for use according to claim 13, wherein said immature hematopoietic cells and said isolated population of cells are autologous or wherein said immature hematopoietic cells are autologous and said isolated population of cells are non-autologous.

15. The isolated population of cells for use according to claim 2 or 13, wherein said anti-third party cells having a central memory T-lymphocyte (Tcm) phenotype, said cells being tolerance-inducing cells and capable of homing to the lymph nodes following transplantation are generated by:

(a) contacting peripheral blood mononuclear cells (PBMC) with a third party antigen or antigens in the presence of IL-21 under conditions

which allow elimination of GVH reactive cells; and
(b) culturing said cells resulting from step (a) in the presence of IL-15 in an antigen free environment under conditions which allow proliferation of cells comprising said central memory T-lymphocyte (Tcm) phenotype, thereby generating the isolated population of cells.

16. The method of claim 1, or isolated population of cells for use according to claim 15, wherein said conditions which allow elimination of GVH reactive cells comprise culturing for 1-5 days.

17. The method of claim 1, or isolated population of cells for use according to claim 15, wherein said culturing in the presence of IL-15 is effected for 3-30 days.

18. The method of claim 1, or isolated population of cells for use according to claim 15, wherein said conditions which allow proliferation of cells comprising said central memory T-lymphocyte (Tcm) phenotype further comprise IL-7 and/or IL-21.

19. The isolated population for use of cells according to claim 2 or 13, wherein said disease comprises leukemia or lymphoma.

Patentansprüche

1. In-Vitro-Verfahren zum Erzeugen einer isolierten Zellpopulation, die Drittzellen umfasst, die keine Wirt-gegen-Transplantat-Reaktion (GVHD - Graft Versus Host Disease) erzeugen und einen zentralen T-Gedächtniszellen-(Tem)-Phenotyp aufweisen, wobei der T-Gedächtniszellen-(Tem)-Phenotyp eine $CD8^+/CD62L^+/CD45RO+$-Signatur umfasst und wobei mindestens 50 % der isolierten Zellpopulation die Signatur aufweisen, wobei die Zellen toleranz-auslösende Zellen sind und in der Lage sind, nach einer Transplantation Lymphknoten als Ziel zu suchen, wobei das Verfahren Folgendes umfasst:

(a) Inkontaktbringen von mononukleären Zellen des peripheren Blutes (PBMC) mit einem Drittantigen oder Drittantigenen in Gegenwart von IL-21 unter Bedingungen, die das Beseitigen GVH-reaktiver Zellen ermöglichen, und
(b) Kultivieren der in Schritt (a) entstandenen Zellen in Gegenwart von IL-15 in einer antigenfreien Umgebung unter Bedingungen, welche die Proliferation von Zellen ermöglichen, die den zentralen T-Gedächtniszellen-(Tem)-Phenotyp umfassen, wodurch die isolierte Zellpopulation erzeugt wird.

2. Isolierte Zellpopulation, Drittzellen umfassend, die keine Wirt-gegen-Transplantat-Reaktion (GVHD - Graft Versus Host Disease) erzeugen und einen zentralen T-Gedächtniszellen-(Tem)-Phenotyp aufweisen, wobei der T-Gedächtniszellen-(Tem)-Phenotyp eine $CD8^+/CD62L^+/CD45RO+$-Signatur umfasst und wobei mindestens 50 % der isolierten Zellpopulation die Signatur aufweisen, wobei die Zellen toleranz-auslösende Zellen sind und in der Lage sind, nach einer Transplantation Lymphknoten als Ziel zu suchen, zur Verwendung als unterstützende Behandlung beim Ausmerzen einer Erkrankung in einem Subjekt, dem nicht gen-identisches Zell- oder Gewebetransplantat transplantiert wurde, wobei die Zellen entweder

(I) nicht gen-identisch mit sowohl dem Subjekt als auch dem Transplantat sind oder
(II) nicht gen-identisch mit dem Transplantat und gen-identisch mit dem Subjekt sind.

3. Isolierte Zellpopulation zur Verwendung nach Anspruch 2, wobei das Zell- oder Gewebetransplantat nicht autolog und die isolierte Zellpopulation autolog ist.

4. Isolierte Zellpopulation zur Verwendung nach Anspruch 2, wobei das Zell- oder Gewebetransplantat und die isolierte Zellpopulation von verschiedenen Spendern stammen.

5. Isolierte Zellpopulation zur Verwendung nach Anspruch 2, wobei die Erkrankung ein Malignom oder optional ein B-Zellen-Malignom ist.

6. Isolierte Zellpopulation zur Verwendung nach Anspruch 2, wobei das Transplantat Knochenmarkzellen und optional unreife blutbildende Zellen umfasst.

7. Isolierte Zellpopulation zur Verwendung nach Anspruch 6, wobei die unreifen blutbildenden Zellen nicht gen-identisch mit dem Subjekt sind und die isolierte Zellpopulation gen-identisch mit dem Subjekt ist.

8. Isolierte Zellpopulation zur Verwendung nach Anspruch 7, wobei die unreifen blutbildenden Zellen nicht autolog und die isolierte Zellpopulation autolog ist.

9. Isolierte Zellpopulation zur Verwendung nach Anspruch 6, wobei die unreifen blutbildenden Zellen nicht gen-identisch mit dem Subjekt sind und die isolierte Zellpopulation nicht gen-identisch mit sowohl dem Subjekt als auch dem Transplantat ist.

10. Isolierte Zellpopulation zur Verwendung nach Anspruch 9, wobei die unreifen blutbildenden Zellen und die isolierte Zellpopulation von verschiedenen

Spendern stammen.

11. Verfahren nach Anspruch 1 oder isolierte Zellpopulation zur Verwendung nach Anspruch 2, wobei die Lymphknoten periphere Lymphknoten oder mesenteriale Lymphknoten sind.

12. Isolierte Zellpopulation zur Verwendung nach Anspruch 2, wobei die Zellen, die nicht gen-identisch mit dem Transplantat und gen-identisch mit dem Subjekt sind, autologe Zellen umfassen.

13. Therapeutisch wirksame Menge einer isolierten Zellpopulation, Drittzellen umfassend, die keine Wirt-gegen-Transplantat-Reaktion (GVHD - Graft Versus Host Disease) erzeugen und einen zentralen T-Gedächtniszellen-(Tem)-Phenotyp aufweisen, wobei der T-Gedächtniszellen-(Tem)-Phenotyp eine $CD8^+/CD62L^+/CD45RO+$-Signatur umfasst und wobei mindestens 50 % der isolierten Zellpopulation die Signatur aufweisen, wobei die Zellen toleranzauslösende Zellen und in der Lage sind, nach einer Transplantation Lymphknoten als Ziel zu suchen, zur Verwendung als unterstützende Behandlung beim Ausmerzen einer Erkrankung in einem Subjekt, dem unreife blutbildende Zellen transplantiert wurden, und wobei ferner die unreifen blutbildenden Zellen wahlweise gen-identisch mit dem Subjekt oder nicht gen-identisch mit dem Subjekt sind.

14. Isolierte Zellpopulation zur Verwendung nach Anspruch 13, wobei die unreifen blutbildenden Zellen und die isolierte Zellpopulation autolog sind oder die unreifen blutbildenden Zellen autolog und die isolierte Zellpopulation nicht autolog ist.

15. Isolierte Zellpopulation zur Verwendung nach Anspruch 2 oder 13, wobei die Drittzellen einen zentralen T-Gedächtniszellen-(Tem)-Phenotyp aufweisen, wobei die Zellen, die toleranz-auslösende Zellen sind und in der Lage sind, nach einer Transplantation Lymphknoten als Ziel zu suchen, durch Folgendes erzeugt werden:

(a) Inkontaktbringen von mononukleären Zellen des peripheren Blutes (PBMC) mit einem Drittantigen oder Drittantigenen in Gegenwart von IL-21 unter Bedingungen, die das Beseitigen GVH-reaktiver Zellen ermöglichen, und
(b) Kultivieren der in Schritt (a) entstandenen Zellen in Gegenwart von IL-15 in einer antigenfreien Umgebung unter Bedingungen, welche die Proliferation von Zellen ermöglichen, die den zentralen T-Gedächtniszellen-(Tem)-Phenotyp umfassen, wodurch die isolierte Zellpopulation erzeugt wird.

16. Verfahren nach Anspruch 1 oder isolierte Zellpopulation zur Verwendung nach Anspruch 15, wobei die Bedingungen, welche die Beseitigung von GVH-reaktiven Zellen ermöglichen, das Kultivieren über 1 bis 5 Tage umfassen.

17. Verfahren nach Anspruch 1 oder isolierte Zellpopulation zur Verwendung nach Anspruch 15, wobei das Kultivieren in Gegenwart von IL-15 3 bis 30 Tage lang durchgeführt wird.

18. Verfahren nach Anspruch 1 oder isolierte Zellpopulation zur Verwendung nach Anspruch 15, wobei die Bedingungen, welche die Proliferation von Zellen ermöglichen, die den zentralen T-Gedächtniszellen-(Tem)-Phenotyp umfassen, ferner IL-7 und/oder IL-21 umfassen.

19. Isolierte Zellpopulation zur Verwendung nach Anspruch 2 oder 13, wobei die Erkrankung Leukämie oder ein Lymphom umfasst.

Revendications

1. Procédé *in vitro* de génération d'une population de cellules isolée comprenant des cellules anti-tiers n'induisant pas de réaction du greffon contre l'hôte (RGCH) ayant un phénotype de lymphocyte T à mémoire centrale (Tcm), ledit phénotype de lymphocyte T à mémoire centrale (Tcm) comprenant une signature $CD8^+/CD62L^+/CD45RO+$ et dans lequel au moins 50 % de la population de cellules isolée possède ladite signature, lesdites cellules étant des cellules induisant une tolérance et capables de domiciliation dans les ganglions lymphatiques suite à une transplantation, le procédé comprenant :

(a) la mise en contact de cellules mononucléaires du sang périphérique (PBMC) avec un antigène ou des antigènes tiers en présence d'IL-21 dans des conditions qui permettent une élimination des cellules induisant une réaction de GCH ; et
(b) la mise en culture desdites cellules résultant de l'étape (a) en présence d'IL-15 dans un environnement dépourvu d'antigène et dans des conditions qui permettent la prolifération de cellules comprenant ledit phénotype de lymphocyte T à mémoire centrale (Tcm), en générant ainsi la population de cellules isolée.

2. Population de cellules isolée comprenant des cellules anti-tiers n'induisant pas de réaction du greffon contre l'hôte (RGCH) ayant un phénotype de lymphocyte T à mémoire centrale (Tcm), ledit phénotype de lymphocyte T à mémoire centrale (Tcm) comprenant une signature $CD8^+/CD62L^+/CD45RO+$ et où au moins 50 % de la population de cellules isolée

possède ladite signature, lesdites cellules étant des cellules induisant une tolérance et capables de domiciliation dans les ganglions lymphatiques suite à une transplantation, destinée à être utilisée en tant que traitement adjuvant pour l'éradication d'une maladie chez un sujet qui a reçu une transplantation d'une greffe de cellules ou de tissu non syngénique, où lesdites cellules sont :

(i) non syngéniques avec à la fois le sujet et ladite greffe ; ou
(ii) non syngéniques avec ladite greffe et syngéniques avec le sujet.

3. Population de cellules isolée destinée à être utilisée selon la revendication 2, où ladite greffe de cellules ou de tissu est non autologue et ladite population de cellules isolée est autologue.

4. Population de cellules isolée destinée à être utilisée selon la revendication 2, où ladite greffe de cellules ou de tissu et ladite population de cellules isolée proviennent de donneurs différents.

5. Population de cellules isolée destinée à être utilisée selon la revendication 2, où ladite maladie comprend une malignité et facultativement une malignité à cellules B.

6. Population de cellules isolée destinée à être utilisée selon la revendication 2, où ladite greffe comprend des cellules de moelle osseuse et facultativement des cellules hématopoïétiques immatures.

7. Population de cellules isolée destinée à être utilisée selon la revendication 6, où lorsque lesdites cellules hématopoïétiques immatures sont non syngéniques avec le sujet, ladite population de cellules isolée est syngénique avec le sujet.

8. Population de cellules isolée destinée à être utilisée selon la revendication 7, où lesdites cellules hématopoïétiques immatures sont non autologues et ladite population de cellules isolée est autologue.

9. Population de cellules isolée destinée à être utilisée selon la revendication 6, où lorsque lesdites cellules hématopoïétiques immatures sont non syngéniques avec le sujet, ladite population de cellules isolée est non syngénique avec à la fois le sujet et avec ladite greffe.

10. Population de cellules isolée destinée à être utilisée selon la revendication 9, où lesdites cellules hématopoïétiques immatures et ladite population de cellules isolée proviennent de donneurs différents.

11. Procédé selon la revendication 1, ou population de cellules isolée destinée à être utilisée selon la revendication 2, où lesdits ganglions lymphatiques comprennent des ganglions lymphatiques périphériques ou des ganglions lymphatiques mésentériques.

12. Population de cellules isolée destinée à être utilisée selon la revendication 2, où lesdites cellules non syngéniques avec ladite greffe et syngéniques avec le sujet comprennent des cellules autologues.

13. Quantité thérapeutiquement efficace d'une population de cellules isolée comprenant des cellules anti-tiers n'induisant pas de réaction du greffon contre l'hôte (RGCH) ayant un phénotype de lymphocyte T à mémoire centrale (Tcm), ledit phénotype de lymphocyte T à mémoire centrale (Tcm) comprenant une signature $CD8^+/CD62L^+/CD45RO+$ et où au moins 50 % de la population de cellules isolée possède ladite signature, lesdites cellules étant des cellules induisant une tolérance et capables de domiciliation dans les ganglions lymphatiques suite à une transplantation, destinée à être utilisée en tant que traitement adjuvant pour l'éradication d'une maladie chez un sujet qui a reçu une transplantation de cellules hématopoïétiques immatures, et en outre où lorsque lesdites cellules hématopoïétiques immatures sont syngéniques avec le sujet, ladite population de cellules isolée est sélectionnée pour être syngénique avec le sujet ou non syngénique avec le sujet.

14. Population de cellules isolée destinée à être utilisée selon la revendication 13, dans laquelle lesdites cellules hématopoïétiques immatures et ladite population de cellules isolée sont autologues ou dans laquelle lesdites cellules hématopoïétiques immatures sont autologues et ladite population de cellules isolée est non autologue.

15. Population de cellules isolée destinée à être utilisée selon la revendication 2 ou 13, dans laquelle lesdites cellules anti-tiers ayant un phénotype de lymphocyte T à mémoire centrale (Tcm), lesdites cellules étant des cellules induisant une tolérance et capables de domiciliation dans les ganglions lymphatiques suite à une transplantation, sont générées par :

(a) la mise en contact de cellules mononucléaires du sang périphérique (PBMC) avec un antigène ou des antigènes tiers en présence d'IL-21 dans des conditions qui permettent une élimination des cellules induisant une réaction de GCH ; et
(b) la mise en culture desdites cellules résultant de l'étape (a) en présence d'IL-15 dans un environnement dépourvu d'antigène et dans des conditions qui permettent la prolifération de cellules comprenant ledit phénotype de lymphocyte T à mémoire centrale (Tcm), en générant ainsi

la population de cellules isolée.

16. Procédé selon la revendication 1, ou population de cellules isolée destinée à être utilisée selon la revendication 15, où lesdites conditions qui permettent une élimination des cellules induisant une réaction de GCH comprennent une culture pendant 1 à 5 jours.

17. Procédé selon la revendication 1, ou population de cellules isolée destinée à être utilisée selon la revendication 15, où ladite mise en culture en présence d'IL-15 est réalisée pendant 3 à 30 jours.

18. Procédé selon la revendication 1, ou population de cellules isolée selon la revendication 15, où lesdites conditions qui permettent la prolifération de cellules comprenant ledit phénotype de lymphocyte T à mémoire centrale (Tcm) comprennent en outre l'IL-7 et/ou l'IL-21.

19. Population de cellules isolée destinée à être utilisée selon la revendication 2 ou 13, où ladite maladie comprend une leucémie ou un lymphome.

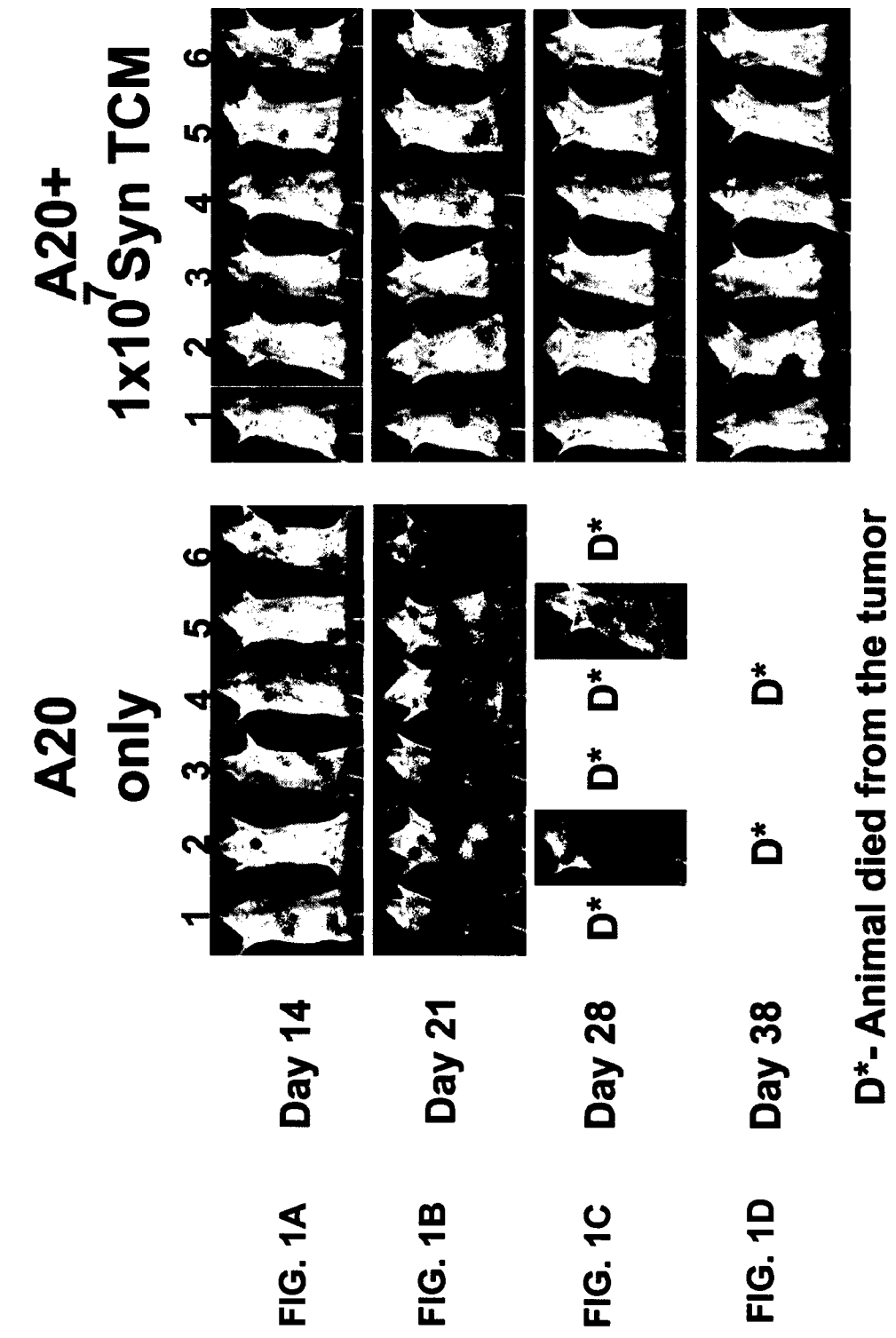
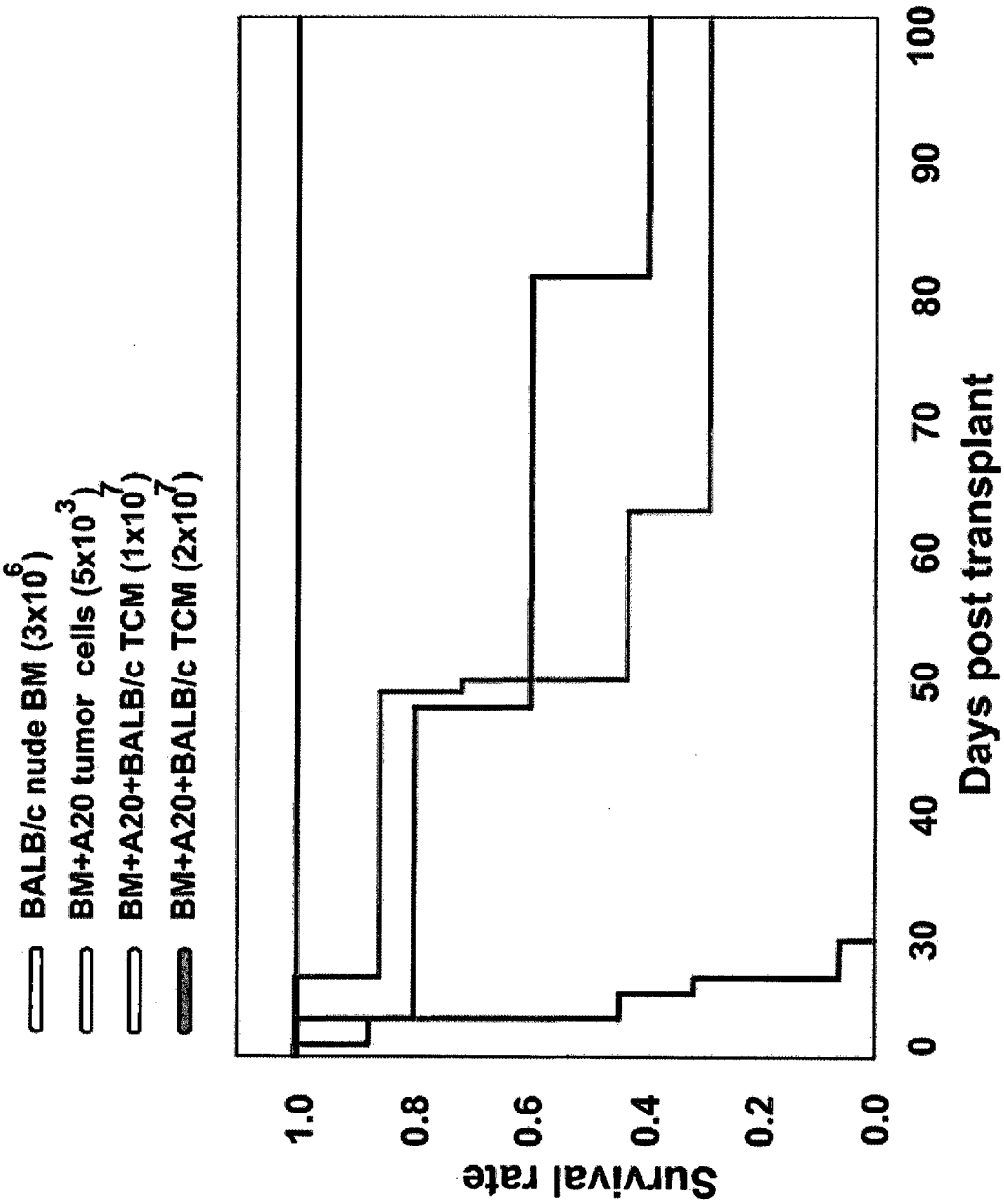


FIG. 1A FIG. 1B FIG. 1C FIG. 1D FIG. 1E FIG. 1F FIG. 1G FIG. 1H

FIG. 1I


BALB/c nude BM (3x10^6)
BM+A20 tumor cells (5x10^3)
BM+A20+BALB/c TCM (1x10^7)
BM+A20+BALB/c TCM (2x10^7)
Survival rate
1.0
0.8
0.6
0.4
0.2
0.0
0
30
40
50
60
70
80
90
100
Days post transplant

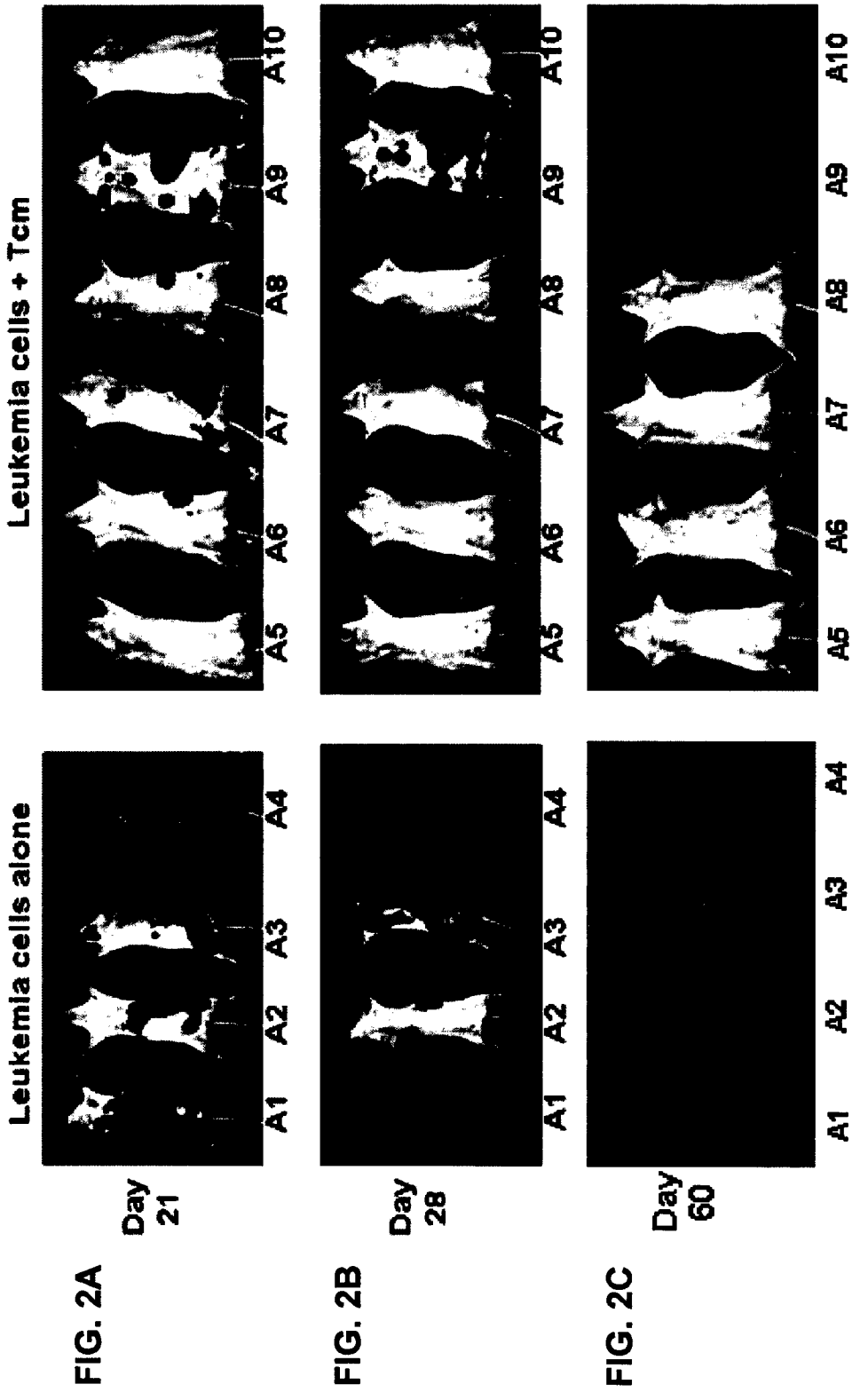
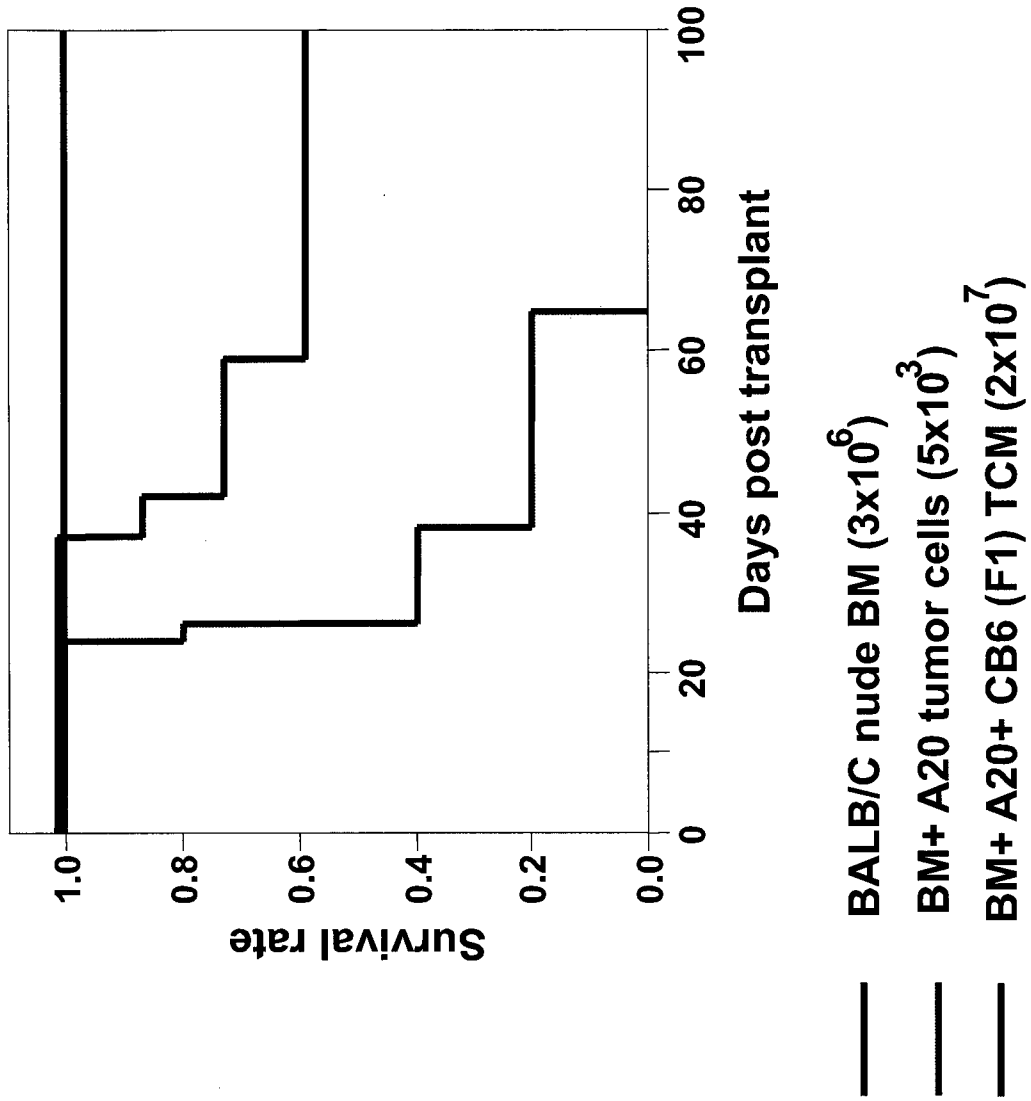


FIG. 2A FIG. 2B FIG. 2C FIG. 2D FIG. 2E FIG. 2F

FIG. 2G


Survival rate
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
Days post transplant
BALB/C nude BM (3x10^6)
BM+ A20 tumor cells (5x10^3)
BM+ A20+ CB6 (F1) TCM (2x10^7)

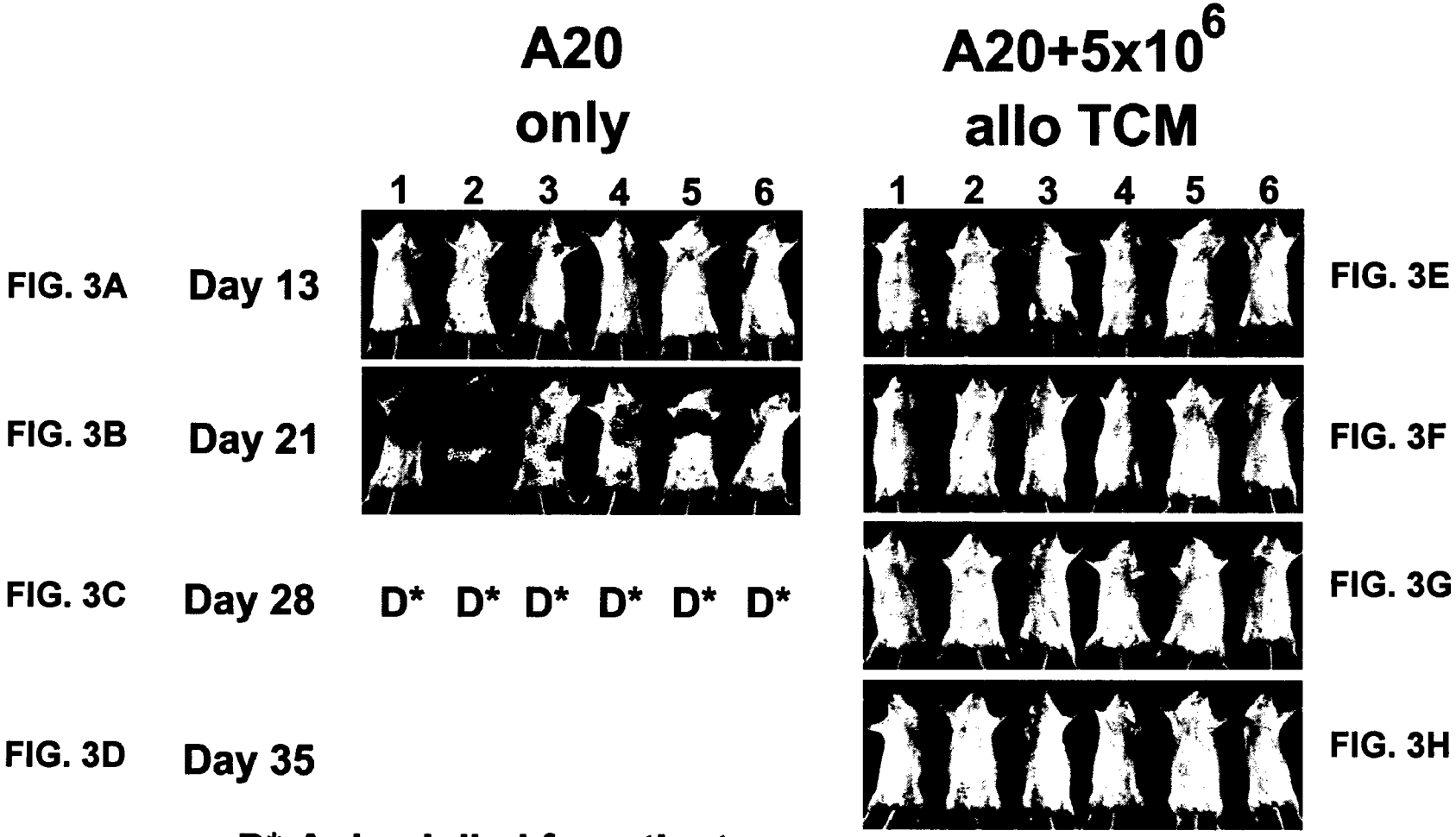
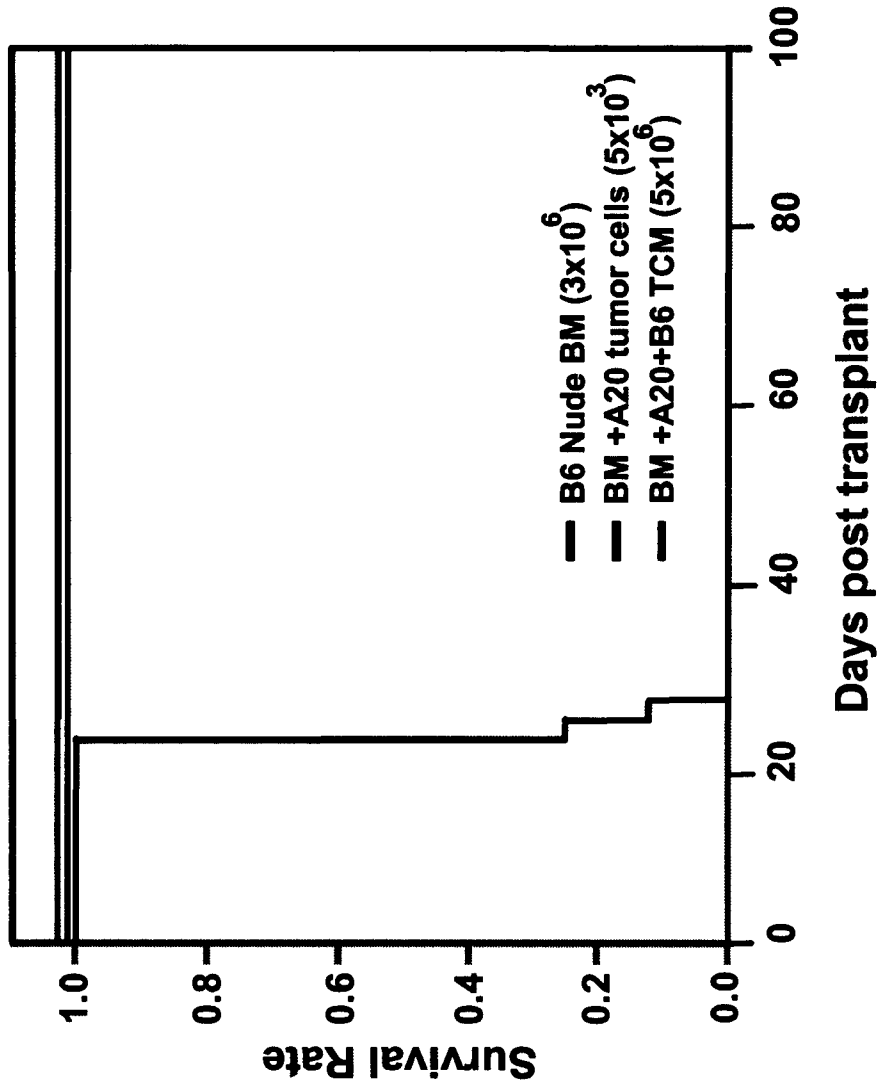

A20
only
A20+5x10^6
allo TCM
1 2 3 4 5 6
1 2 3 4 5 6
FIG. 3A
Day 13
FIG. 3E
FIG. 3B
Day 21
FIG. 3F
FIG. 3C
Day 28
D* D* D* D* D* D*
FIG. 3G
FIG. 3D
Day 35
FIG. 3H
D*-Animal died from the tumor

FIG. 3I


Survival Rate
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
Days post transplant
B6 Nude BM (3x10^6)
BM +A20 tumor cells (5x10^3)
BM +A20+B6 TCM (5x10^6)

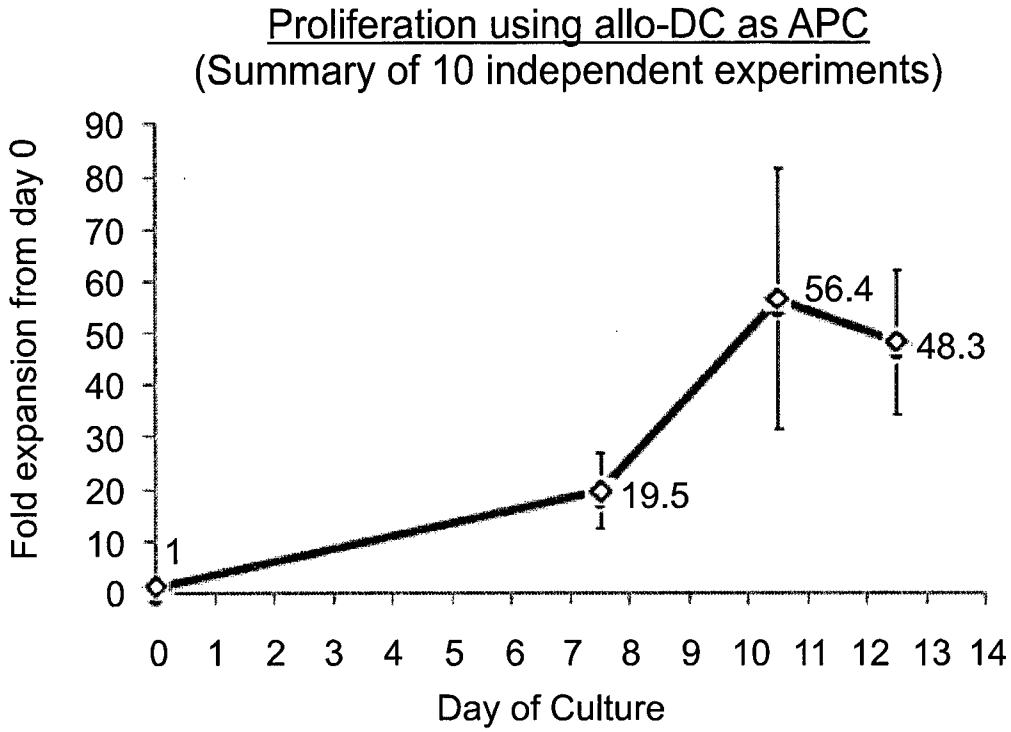

Proliferation using allo-DC as APC
(Summary of 10 independent experiments)
Fold expansion from day 0
90
80
70
60
50
40
30
20
10
0
1
19.5
56.4
48.3
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Day of Culture

FIG. 4A

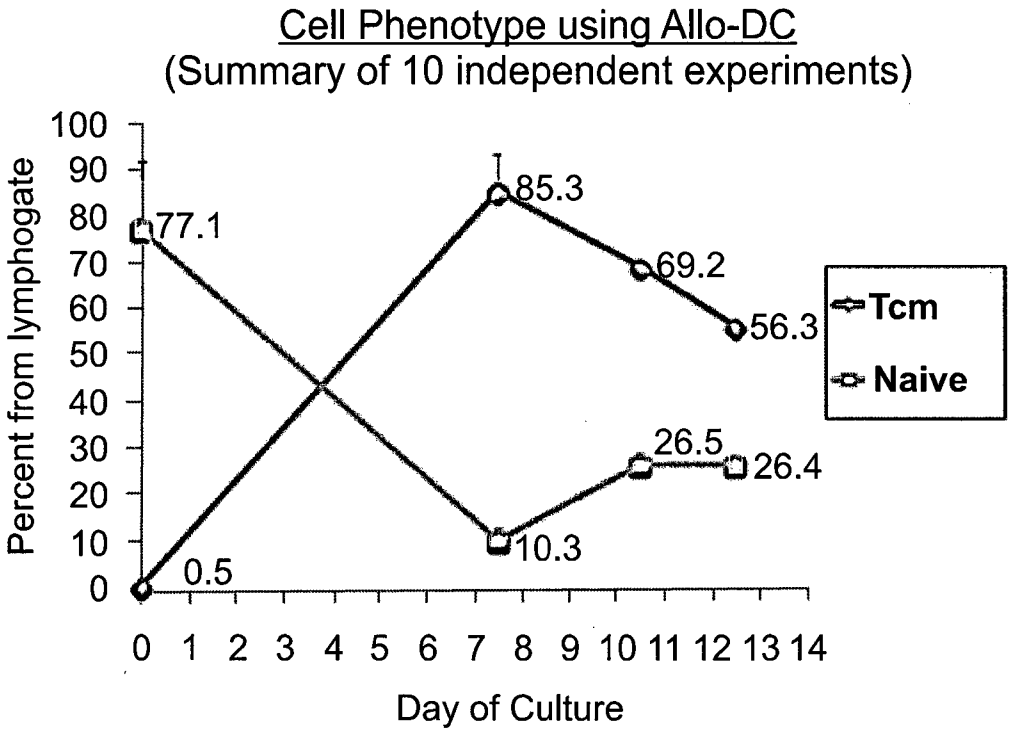

Cell Phenotype using Allo-DC
(Summary of 10 independent experiments)
Percent from lymphogate
100
90
80
70
60
50
40
30
20
10
0
77.1
85.3
69.2
56.3
0.5
10.3
26.5
26.4
Tcm
Naive
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Day of Culture

FIG. 4B

FIG. 5

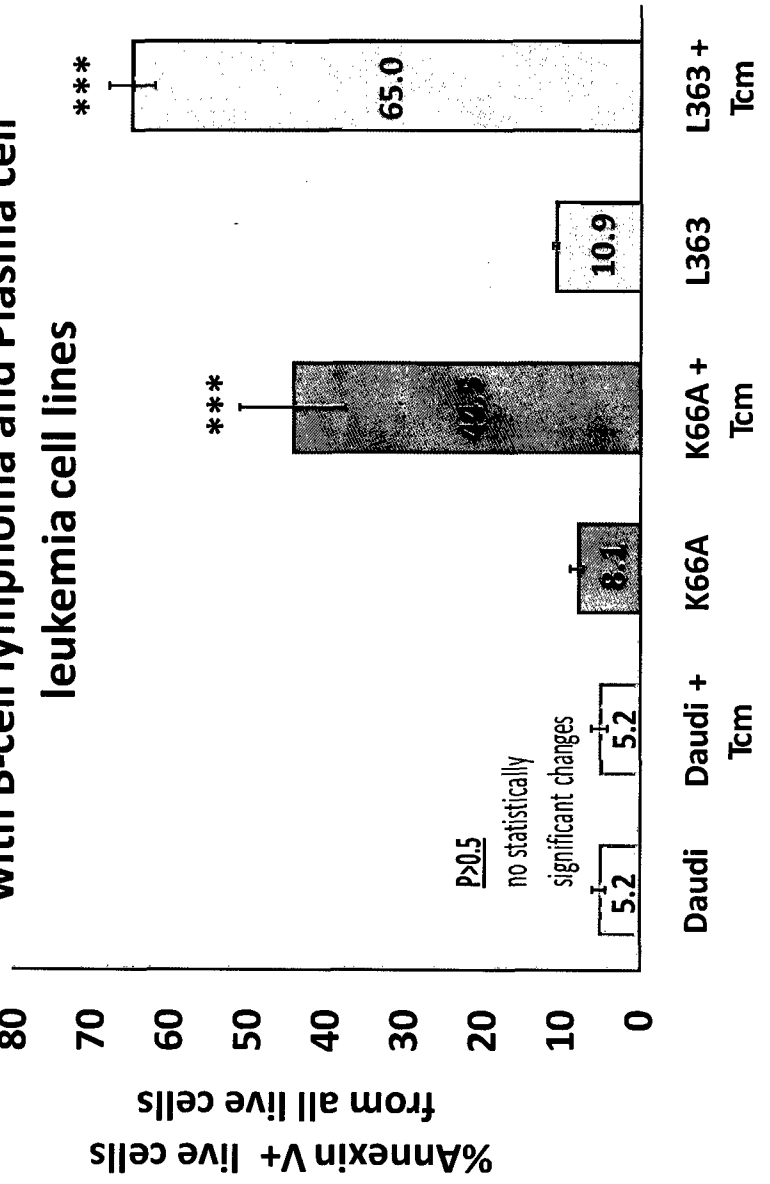
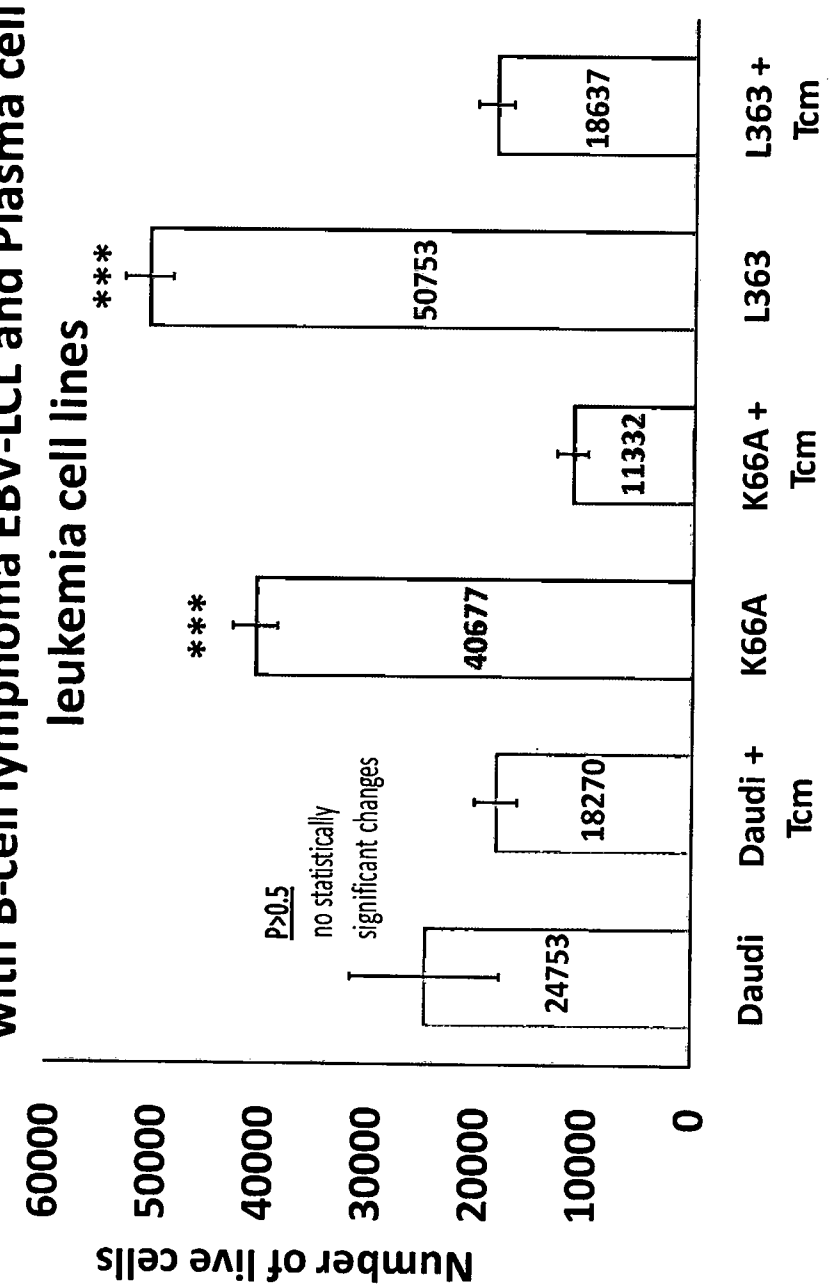

% Apoptotic cells after 22h of MLR with B-cell lymphoma and Plasma cell leukemia cell lines
%Annexin V+ live cells from all live cells
0
10
20
30
40
50
60
70
80
5.2
5.2
8.1
***
***
65.0
10.9
P>0.5
no statistically significant changes
Daudi
Daudi + Tcm
K66A
K66A + Tcm
L363
L363 + Tcm

FIG. 6


Number of live cells after 22h of MLR with B-cell lymphoma EBV-LCL and Plasma cell leukemia cell lines
Number of live cells
60000
50000
40000
30000
20000
10000
0
***
***
P>0.5
no statistically significant changes
24753
18270
40677
50753
11332
18637
Daudi
Daudi + Tcm
K66A
K66A + Tcm
L363
L363 + Tcm

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EP2613801

ANTI-HARMADIK FÉL KÖZPONTI MEMÓRIA T-SEJTEK ALKALMAZÁSA LEUKÉMIA/LIMFÓMA-ELLENES KEZELÉSHEZ

SZABADALMI IGÉNYPONTOK

1. In vitro eljárás olyan sejtek izolált populációjának kialakítására, amelyek tartalmaznak nem-graft (beültetett), gazdaszervezet ellen [non-graft versus host (GVBH)] indukáló anti-harmadik fél sejteket (anti-third party cell), amelyeknek központi memória T-limfocita (Tcm) fenotípusa van, ahol az említett központi memória T-limfocita (Tcm) fenotípus $CD8^+/CD62L^+/CD45RO^+$ szignatúrát (ismertetőjelet) tartalmaz, és ahol a sejtek izolált populációjának legalább 50%-a rendelkezik az említett szignatúrával, ahol az az említett sejtek tűrés-indukáló sejtek (tolerance-inducing cell) és képesek a transzplantációt követően hozzáirányulni (homing) a nyirokcsomókhoz; az eljárás a következő lépéseket tartalmazza:

(a) érintkezésbe hozunk perifériás vér mononukleáris sejteket [peripheral blood mononuclear cells (PBMC)] egy harmadik-fél antigénnel vagy antigénekkel IL-21 jelenlétében olyan körülmények között, amelyek lehetővé teszik a GVH-reaktív sejtek eltüntetését; és

(b) tenyésztjük az (a) lépésből keletkezett említett sejteket IL-15 jelenlétében antigén-mentes környezetben olyan körülmények között, amelyek lehetővé teszik azon sejtek burjánzását (proliferation), amelyek tartalmazzák az említett központi memória T-limfocita (Tcm) fenotípust, ezáltal alakítva ki a sejtek izolált populációját.

2. Sejtek izolált populációja, amely tartalmaz nem-graft (beültetett), gazdaszervezet ellen [non-graft versus host (GVBH)] indukáló anti-harmadik fél sejteket (anti-third party cell), amelyeknek központi memória T-limfocita (Tcm) fenotípusa van, ahol az említett központi memória T-limfocita (Tcm) fenotípus $CD8^+/CD62L^+/CD45RO^+$ szignatúrát (ismertetőjelet) tartalmaz, és ahol a sejtek izolált populációjának legalább 50%-a rendelkezik az említett szignatúrával, ahol az az említett sejtek tűrés-indukáló sejtek (tolerance-inducing cell) és képesek a transzplantációt követően hozzáirányulni (homing) a nyirokcsomókhoz; az izolált populáció adjuváns kezeléshez szolgál egy betegség megsemmisítéséhez egy olyan betegben, aki transzplantáción esett át egy nem-veleszületeti (non-syngeneic) sejttel vagy szövet grafttal, ahol az említett sejtek vagy

(i) nem-veleszületeitek (non-syngeneic) mind a beteg (subject), mind az említett graft szempontjából; vagy

(ii) nem-veleszületettek az említett graft szempontjából, de veleszületeitek (syngeneic) a beteg szempontjából.

3. Sejtek 2. igénypont szerinti izolált populációja alkalmazásra, ahol az említett sejt vagy szöveti graft nem-autológ, míg a sejteknek említett izolált populációja autológ.

4. Sejtek 2. igénypont szerinti izolált populációja alkalmazásra, ahol az említett sejt vagy szöveti graft és a sejteknek említett izolált populációja különböző donorokból való.

5. Sejtek 2. igénypont szerinti izolált populációja alkalmazásra, ahol az említett betegség tartalmaz rosszindulatúságot (malignancy), elsősorban B sejt rosszindulatúságot.

6. Sejtek 2. igénypont szerinti izolált populációja alkalmazásra, ahol az említett graft tartalmaz csontvelő sejteket és kívánt esetben éretlen (immature) vérképző (haematopoietic) sejteket.

7. Sejtek 6. igénypont szerinti izolált populációja alkalmazásra, ahol amikor az említett éretlen vérképző sejtek nem-veleszületettek a beteggel (subject), a sejtek említett izolált populációja veleszületett a beteggel.

8. Sejtek 7. igénypont szerinti izolált populációja alkalmazásra, ahol az említett éretlen vérképző sejtek nem-veleszületettek, míg a sejtek említett izolált populációja veleszületett.

9. Sejtek 6. igénypont szerinti izolált populációja alkalmazásra, ahol amikor az említett éretlen vérképző sejtek nem-veleszületettek a beteggel, a sejtek izolált populációja nem-veleszületett sem a beteggel, sem az említett grafttal.

10. Sejtek 9. igénypont szerinti izolált populációja alkalmazásra, ahol az említett éretlen vérképző sejtek és a sejteknek

említett izolált populációja különböző donorokból való.

11. Az 1. igénypont szerinti eljárás, vagy sejtek 2. igénypont szerinti izolált populációja alkalmazásra, ahol az említett nyirokcsomók tartalmaznak kerületi (peripheral) nyirokcsomókat vagy bélfodri (mesenteric) nyirokcsomókat.

12. Sejtek 2. igénypont szerinti izolált populációja alkalmazásra, ahol az említett sejtek, amelyek nem-veleszületettek az említett grafttal és veleszületettek a beteggel, tartalmaznak autológ sejteket.

13. Olyan sejtek izolált populációjának terápiásan hatékony mennyisége, amelyek tartalmaznak nem-graft (beültetett), gazdaszervezet ellen [non-graft versus host (GVBH)] indukáló anti-harmadik fél sejteket (anti-third party cell), amelyeknek központi memória T-limfocita (Tcm) fenotípusa van, ahol az említett központi memória T-limfocita (Tcm) fenotípus CD8+/CD62L+/CD45RO+ szignatúrát (ismertetőjelet) tartalmaz, és ahol a sejtek izolált populációjának legalább 50%-a rendelkezik az említett szignatúrával, ahol az az említett sejtek tűrés-indukáló sejtek (tolerance-inducing cell) és képesek a transzplantációt követően hozzáirányulni (homing) a nyirokcsomókhoz; ez adjuváns kezeléshez szolgál egy betegség megsemmisítéséhez egy olyan betegben, aki transzplantáción esett át éretlen vérképző sejtekkel, és továbbá ahol amikor az éretlen vérképző sejtek veleszületettek a beteggel, a sejtek említett izolált populációja lehet úgy kiválasztva, hogy veleszületett (syngeneic) a beteggel vagy nem-veleszületett (non-syngeneic) a beteggel.

14. Sejtek 13. igénypont szerinti izolált populációja alkalmazásra, ahol az említett éretlen vérképző sejtek és sejtek említett izolált populációja autológok, vagy ahol az említett éretlen vérképző sejtek autológok, míg a sejtek említett izolált populációja nem-autológ.

15. Sejtek 2. vagy 13. igénypont szerinti izolált populációja alkalmazásra, ahol az említett anti-harmadik fél sejtek (anti-third party cell) központi memória T-limfocita (Tcm) fenotípussal rendelkeznek, ahol az említett sejtek tűrés-indukáló sejtek (tolerance-inducing cell) és képesek a transzplantációt követően hozzáirányulni (homing) a nyirokcsomókhoz, és a következő lépések révén vannak kialakítva:

(a) érintkezésbe hozunk perifériás vér mononukleáris sejteket [peripheral blood mononuclear cells (PBMC)] egy harmadik-fél antigénnel vagy antigénekkel IL-21 jelenlétében olyan körülmények között, amelyek lehetővé teszik a GVH-reaktív sejtek eltűntetését; és

(b) tenyésztjük az (a) lépésből keletkezett említett sejteket IL-15 jelenlétében antigén-mentes környezetben olyan körülmények között, amelyek lehetővé teszik azon sejtek burjánzását (proliferation), amelyek tartalmazzák az említett központi memória T-limfocita (Tcm) fenotípust, ezáltal alakítva ki a sejtek izolált populációját.

16. Az 1. igénypont szerinti eljárás, vagy a sejtek 15. igénypont szerinti populációja alkalmazásra, ahol azok az említett körülmények, amelyek lehetővé teszik a GVH-reaktív sejtek eltávolítását, tartalmazzák a tenyésztést 1-5 napon keresztül.

17. Az 1. igénypont szerinti eljárás, vagy a sejtek 15. igénypont szerinti populációja alkalmazásra, ahol az említett tenyésztést IL-15 jelenlétében 3-30 napon át végezzük.

18. Az 1. igénypont szerinti eljárás, vagy a sejtek 15. igénypont szerinti populációja alkalmazásra, ahol azok az említett körülmények, amelyek lehetővé teszik azon sejtek burjánzását, amelyek tartalmazzák az említett központi memória T-limfocita (Tcm) fenotípust, tartalmaznak továbbá IL-7-et és/vagy IL-21-et.

19. Sejtek 2. vagy 13. igénypont szerinti izolált populációja alkalmazásra, ahol az említett betegség tartalmaz leukémiát vagy limfómát.