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CELLULES SOUCHES
(54) Title: METHODS FOR USING ALDHbr CELLS TO SUPPLEMENT STEM CELL TRANSPLANTATION

(57) **Abrégé/Abstract:**

The present invention relates to methods repairing, regenerating, and reconstituting tissues by transplanting at least two stem cell populations, wherein the first and the second population of stem cells are introduced into a subject separated by a time interval of about 2 to about 24 hours. The first population comprises stem cells derived from umbilical cord. The second population comprises ALDHbr cells. These ALDHbr cells can be administered to a patient immediately after isolation or can be primed in culture using a combination of cytokines for about 2 to about 7 days prior to transplantation. The methods of the invention are useful in accelerating time to neutrophil and/or platelet engraftment and immune reconstitution following myeloablative therapy.



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(54) Title: METHODS FOR USING ALDHBR CELLS TO SUPPLEMENT STEM CELL TRANSPLANTATION

(57) Abstract: The present invention relates to methods repairing, regenerating, and reconstituting tissues by transplanting at least two stem cell populations, wherein the first and the second population of stem cells are introduced into a subject separated by a time interval of about 2 to about 24 hours. The first population comprises stem cells derived from umbilical cord. The second population comprises ALDHbr cells. These ALDHbr cells can be administered to a patient immediately after isolation or can be primed in culture using a combination of cytokines for about 2 to about 7 days prior to transplantation. The methods of the invention are useful in accelerating time to neutrophil and/or platelet engraftment and immune reconstitution following myeloablative therapy.

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5 METHODS FOR USING ALDH^{br} CELLS TO SUPPLEMENT
STEM CELL TRANSPLANTATION

FIELD OF THE INVENTION

10 The present invention relates to improved methods of reconstituting, repairing,
and regenerating tissue using populations of stem cells enriched for early progenitor cells.

BACKGROUND OF THE INVENTION

Over the past decade, umbilical cord blood (UCB) transplantation has been shown
15 to be a viable alternative donor stem cell source for hematopoietic cell transplantation in
subjects with catastrophic diseases treatable with transplantation therapy. UCB cells can
cross partially mismatched HLA barriers without intolerable acute or chronic Graft-
versus-Host Disease (GvHD) (Wagner *et al.* (1996) *Blood* 88(3):795-802; Rubinstein *et*
al. (1998) *N Engl J Med* 339(22):1565-1577; Rocha, *et al.* (2000) *N Engl J Med*
20 342(25):1846-1854) Thus, many subjects lacking a sufficiently matched, living related or
unrelated bone marrow or adult stem cell donor, can use partially HLA-matched UCB
cells for stem cell rescue after myeloablative irradiation and/or chemotherapy. UCB cell
dose, expressed per kilogram of recipient body weight, is the best predictor of outcomes
after UCB transplantation (Kurtzberg J, et al. (1996) *N Engl J Med* 335:157-166; Stevens
25 et al. (2002) *Blood* 100(7):2662-2664). Cell dose thresholds strongly correlating with
outcomes have been identified. In subjects receiving lower cell doses, while durable
engraftment will ultimately occur, there are significant delays in myeloid and platelet
engraftment which, at best, result in longer hospitalization and significant increases in
resource utilization and in the worst cases, result in increased early deaths from infection
30 and regimen-related toxicity.

In infants and children weighing <40kg, it is possible to find a sufficiently
matched UCB unit that will deliver a dose of cells critical for successful engraftment
(defined as 3×10^7 nucleated cells/kg) within a reasonable time frame in >90% of
subjects. In teenagers and adults weighing >40kg, this is possible 30-50% of the time.

Because UCB units contain a relatively fixed number of total nucleated cells, units delivering optimal cell dosing for subjects weighing >70kg will only be identified <15% of the time. Attempts to increase the dose of cells available for UCBT have included *ex vivo* expansion and combined unit transplantation. While expansion of UCB cells *ex vivo* is possible, previous phase I studies of infusion of expanded cells have not resulted in shortening of engraftment times (Jaroscak *et al.* (2003) *Blood* 101(12):5061-5067; McNiece *et al.* (2004) *Cytotherapy* 6(4):311-317). Likewise, combinations of up to 5 UCB units for a single myeloablative transplant have not shortened time to neutrophil or platelet engraftment.

Several strategies have tried to address ways to increase cells available for transplantation with the intent of shortening the time to neutrophil and/or platelet engraftment. If successful, these approaches would increase the safety of the transplant procedure by lessening regimen-related toxicity. Engraftment after UCBT is a major predictor of overall and event free survival. An intervention that could facilitate engraftment by decreasing time to absolute neutrophil count (ANC) recovery and/or overall probability of engraftment would be advantageous.

SUMMARY OF THE INVENTION

Methods are provided herein for use in reconstituting, repairing and regenerating tissue in a subject in need thereof by introducing into the subject at least a first and a second population of cells. The first stem cell population comprises stem cells derived from umbilical cord. The second population of stem cells comprises aldehyde dehydrogenase positive (ALDH^{br}) cells isolated from umbilical cord wherein the cells are either used without further manipulation following isolation or are primed in culture using a combination of cytokines for about 2 to about 7 days prior to introducing the cells into the subject. The second cell population is introduced into the subject between 2 and 24 hours after introduction of the first population of UCB.

The methods of the invention are particularly useful in accelerating time to neutrophil and/or platelet engraftment and immune reconstitution following myeloablative therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1-4 show interim results for neutrophil engraftment and platelet engraftment for 14 patients undergoing the UCB transplant procedures described herein

(labeled "ALDH^{br}"). Patients were enrolled at various timepoints, and the trial is ongoing. Therefore, at the time of the analysis, some of the patients had not reached the engraftment endpoints demonstrated in these figures.

5 Figure 1 shows the cumulative incidence of neutrophil engraftment up to day 60 in the treatment group compared to historical controls of 69 patients treated for metabolic diseases in the COBLT study. Neutrophil engraftment was defined as reaching an ANC of at least 500 neutrophils/ μ l.

10 Figure 2 shows the preliminary cumulative incidence of neutrophil engraftment up to day 60 for 14 patients in the treatment group compared to historical controls of 191 patients treated for malignant diseases in the COBLT study. Neutrophil engraftment was defined as reaching an ANC of at least 500 neutrophils/ μ l.

15 Figure 3 shows the preliminary cumulative incidence of platelet engraftment up to day 200 for 14 patients in the treatment group compared to historical controls of 69 patients treated for metabolic diseases in the COBLT study. Platelet engraftment was defined as maintaining a platelet count of at least 50,000 platelets/ μ l of blood without transfusion support.

20 Figure 4 shows the preliminary cumulative incidence of platelet engraftment up to day 200 for 14 patients in the treatment group compared to historical controls of 191 patients treated for malignant diseases in the COBLT study. Platelet engraftment was defined as maintaining a platelet count of at least 50,000 platelets/ μ l of blood without transfusion support.

25 DETAILED DESCRIPTION OF THE INVENTION

I. Overview

30 Stem and progenitor cells (SPC) reproduce and maintain developmental potential until specific biological signals induce the cells to differentiate into a specific cell type or tissue type. Adult stem and progenitor cells (ASPC) are small populations of SPC that remain in tissues of an organism following birth and are continuously renewed during a lifetime. As used herein, "stem cell" refers to a cell with the capability of differentiation and self-renewal, as well as the capability to regenerate tissue. As used herein,

"engraftment" and "in vivo regeneration" refer to the biological process in which implanted or transplanted stem cells reproduce themselves and/or produce differentiated cell progeny in a host organism, and/or replace lost or damaged cells in the host.

Allogeneic cell therapy is used to treat a variety of diseases or pathological conditions. Allogeneic cell therapy is an important curative therapy for several types of malignancies and viral diseases. Allogeneic cell therapy involves the infusion or transplant of cells to a subject, whereby the infused or transplanted cells are derived from a donor other than the subject. As used herein, the term "derive" or "derived from" is intended to obtain physical or informational material from a cell or an organism of interest, including isolation from, collection from, and inference from the organism of interest.

Types of allogeneic donors that have been utilized for allogeneic cell therapy protocols include: human leukocyte antigen (HLA)-matched siblings, matched biologically unrelated donors, partially matched biologically related donors, biologically related umbilical cord blood donors, and biologically unrelated umbilical cord blood donors. The allogeneic donor cells are usually obtained by bone marrow harvest, collection of peripheral blood or collection of placental cord blood at birth.

The methods of the present invention encompass the administration or introduction of two cell preparations (or "populations"), wherein the administration of each is separated in time so as to accelerate hematopoiesis. "Administration" or "introduction" refers to the intravenous introduction of the cell populations described herein into a subject. In some embodiments, the administration of the two cell preparations follows myeloablative therapy.

For the purposes of the present invention, one cell preparation is referred to as the "first cell population" and the other cell preparation is referred to as the "second cell population" or "supplement cell population." The second cell population is administered to a subject no more than about 1 hour, no more than about 1.5 hours, about 2 hours, about 2.5 hours, about 3 hours, about 3.5 hours, about 4 hours, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, or no more than about 24 hours after the first cell population.

The first population comprises umbilical cord blood cells. The second cell population comprises SPC that are ALDH^{br}, and thus contain most or all of the stem cells

present in a stem cell source. The first and the second cell population may be obtained or derived from the same or different donors. Where the first and second cell populations are derived from the same donor, the UCB collected from the donor can be apportioned into about an 80%/20%, about a 75%/25%, about a 60%/40%, about a 65%/35%, about a 60%/40%, about a 55%/45%, or about a 50%/50% split for the first and second cell populations, respectively. This split can be an apportionment of one batch of cells collected at a particular time (e.g., a single cord unit collected from the donor, split according to the parameters above), or it can be an apportionment of pooled cord blood units collected from one or more donors. The number of nucleated cells required for each infusion is discussed elsewhere herein.

In one embodiment, the ALDH^{br} second population of cells is "primed" prior to introducing the cells into a subject. By "primed" or "priming" is intended that the cells are exposed to cytokines for about 2 to about 7 days before transplantation. In specific embodiments, the cells are primed in culture for about 5 days prior to transplantation in serum free culture medium containing SCF, IL-7, and FLT-3.

Thus, the compositions of the present invention comprising a first and a second population of cells derived from umbilical cord blood are useful in a method of reconstituting blood tissue or other stem and progenitor cell function, wherein the method comprises introducing the second population of cells into a subject in need thereof between 2 and 24 hours after the first population of cells. In these and other embodiments, at least the second population is an enriched ALDH^{br} stem cell population.

II. Indications

The cell populations described herein can be used for a wide variety of treatment protocols in which a tissue or organ of the body is augmented, repaired or replaced by the engraftment, transplantation or infusion of these cell populations. As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results (i.e., "therapeutic response"). For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilization (i.e., not worsening) of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment or receiving different treatment (i.e., only a single dose of cells, or multiple doses of cells spaced greater than

24 hours apart, or some other treatment not encompassed herein). "Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. "Alleviating" a disease means that the extent and/or undesirable clinical manifestations of a disease state are lessened and/or the time course of the progression is slowed or shortened, as compared to a situation without treatment or a different treatment. Typically, the "treatment" entails administering additively effective SPC to the subject to regenerate tissue (particularly hematopoietic cells).

The cell populations useful in the methods described herein may be utilized in a variety of contexts. In one embodiment, the cells may be administered to subjects who have decreased hematologic function resulting from one or more diseases, treatments, or a combination thereof, to accelerate hematologic recovery.

For example, the methods of the invention are useful for the treatment of patients having: diseases resulting from a failure or dysfunction of normal blood cell production and maturation, hyperproliferative stem cell disorders, aplastic anemia, pancytopenia, thrombocytopenia, red cell aplasia, Blackfan-Diamond syndrome due to drugs, radiation, or infection, idiopathic; hematopoietic malignancies, including acute lymphoblastic (lymphocytic) leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, acute malignant myelosclerosis, multiple myeloma, polycythemia vera, agnogenic myelometaplasia, Waldenstrom's macroglobulinemia, Hodgkin's lymphoma, non-Hodgkins's lymphoma; immunosuppression in subjects with malignant, solid tumors, including malignant melanoma, carcinoma of the stomach, ovarian carcinoma, breast carcinoma, small cell lung, carcinoma, retinoblastoma, testicular carcinoma, glioblastoma, rhabdomyosarcoma, neuroblastoma, Ewing's sarcoma, lymphoma; autoimmune diseases, rheumatoid arthritis, diabetes type I, chronic hepatitis, multiple sclerosis, and systemic lupus erythematosus; genetic (congenital) disorders, anemias, familial aplastic, Fanconi's syndrome, Bloom's syndrome, pure red cell aplasia (PRCA), dyskeratosis congenital, Blackfan-Diamond syndrome, congenital dyserythropoietic syndromes I-IV, MPS I, MPS II, MPS III, MPS IV, MPS V, Infantile Krabbe disease, adrenoleukodystrophy, metachromatic leukodystrophy, Tay Sachs disease, Chwachmann-Diamond syndrome, dihydrofolate reductase deficiencies, formamino transferase deficiency, Lesch-Nyhan syndrome, congenital spherocytosis, congenital elliptocytosis, congenital stomatocytosis, congenital Rh null disease, paroxysmal nocturnal hemoglobinuria, G6PD (glucose-6-phosphate dehydrogenase),

variants 1,2,3, pyruvate kinase deficiency, congenital erythropoietin sensitivity, deficiency, sickle cell disease and trait, thalassemia alpha, beta, gamma met-hemoglobinemia, congenital disorders of immunity, severe combined immunodeficiency disease, (SCID), bare lymphocyte syndrome, ionophore-responsive combined, immunodeficiency, combined immunodeficiency with a capping abnormality, nucleoside phosphorylase deficiency, granulocyte actin deficiency, infantile agranulocytosis, Gaucher's disease, adenosine deaminase deficiency, Kostmann's syndrome, reticular dysgenesis, congenital leukocyte dysfunction syndromes; osteopetrosis, myelosclerosis, acquired hemolytic anemias, acquired immunodeficiencies, disorders involving disproportions in lymphoid cell sets and impaired immune functions due to aging phagocyte disorders, Kostmann's agranulocytosis, chronic granulomatous disease, Chediak-Higachi syndrome, neutrophil actin deficiency, neutrophil membrane GP-180 deficiency, metabolic storage diseases, mucopolysaccharidoses, mucopolipidoses, miscellaneous disorders involving immune mechanisms, Wiskott-Aldrich Syndrome, and alpha 1-antitrypsin deficiency.

It has also been shown that the hematologic toxicity sequelae observed with multiple cycles of high-dose chemotherapy is relieved by conjunctive administration of autologous hematopoietic stem cells. Thus, the present method is useful for diseases for which reinfusion of stem cells following myeloablative chemotherapy has been described including acute leukemia, Hodgkin's and non-Hodgkin's lymphoma, neuroblastoma, testicular cancer, breast cancer, multiple myeloma, thalassemia, and sickle cell anemia (Cheson *et al.* (1989) *Ann. Intern. Med.* 30 110:51; Wheeler *et al.* (1990) *J. Clin. Oncol.* 8:648; Takvorian *et al.* (1987) *N. Engl. J. Med.* 316:1499; Yeager, *et al.* (1986) *N. Eng. J. Med.* 315:141; Biron *et al.* (1985) In *Autologous Bone Marrow Transplantation: Proceedings of the First International Symposium*, Dicke *et al.*, eds., p. 203; Peters (1985) *ABMT*, *id.* at p. 189; Barlogie, (1993) *Leukemia* 7:1095; Sullivan, (1993) *Leukemia* 7:1098-1099).

Most chemotherapy agents used to target and destroy cancer cells act by killing all proliferating cells, i.e., cells going through cell division. Since bone marrow is one of the most actively proliferating tissues in the body, hematopoietic stem cells are frequently damaged or destroyed by chemotherapy agents and in consequence, blood cell production is diminishes or ceases. Thus, the present invention is useful for improving myeloablative transplant outcomes by accelerating platelet and neutrophil engraftment following chemotherapy.

III. Source of cell preparations

The methods of the invention generally encompass the use of allogeneic stem cell therapy. Allogeneic cell therapy is an important curative therapy for several types of malignancies and viral diseases. Allogeneic cell therapy involves the infusion or transplant of cells to a subject, whereby the infused or transplanted cells are derived from a donor other than the subject. Types of allogeneic donors that have been utilized for allogeneic cell therapy protocols include: HLA-matched siblings, matched unrelated donors, partially matched family member donors, related umbilical cord blood donors, and unrelated umbilical cord blood donors. The allogeneic donor cells are usually obtained by bone marrow harvest, collection of peripheral blood or collection of placental cord blood at birth.

Allogeneic cells preferably are chosen from human leukocyte antigen (HLA)-compatible donors. Generally, HLA-compatible lymphocytes may be taken from a fully HLA-matched relative such as a parent, brother or sister. However, donor lymphocytes may be sufficiently HLA-compatible with the recipient to obtain the desired result even if a sibling donor is single-locus mismatched. If a donor is unrelated to the recipient, preferably the donor lymphocytes are fully HLA matched with the recipient. In one embodiment, the cells will be obtained from a donor that is HLA-matched at 6/6 loci. In another embodiment, the cells will be obtained from a donor that is HLA-matched at 5/6 loci. In yet another embodiment, the cells will be obtained from a donor that is HLA-matched at 4/6 loci. Mismatches at the A locus are preferred over mismatches at the B locus, which are preferred over mismatches at the DR locus. In various embodiments utilizing UCB, it may not be necessary to HLA-type the cells prior to administration

Thus, in one embodiment, the invention provides a method of treating an individual comprising administering to the individual a first and a second population of SPC collected from at least one donor. "Donor" in this context means an adult, child, infant, or a placenta. In another embodiment, the method comprises administering to the individual a first and/or a second population of SPC that has been collected from a plurality of donors and pooled. Alternatively, the first and the second population of SPC may be taken from multiple donors separately, and administered separately, e.g., one or more donors is used for the first cell population, and one or more of the same or different donors is used for the second cell population.

IV. Collection Methods

Umbilical cord blood may be collected in any medically or pharmaceutically-acceptable manner. Various methods for the collection of cord blood have been described. See, e.g., Coe, U.S. Pat. No. 6,102,871; Haswell, U.S. Pat. No. 6,179,819 B1. UCB may
5 be collected into, for example, blood bags, transfer bags, or sterile plastic tubes. UCB or stem cells derived therefrom may be stored as collected from a single individual (i.e., as a single unit) for administration, or may be pooled with other units for later administration.

If frozen, the cells are transferred to an appropriate cryogenic container and the container decreased in temperature to generally from -120°C to -196°C and maintained at
10 that temperature. When needed, the temperature of the cells (i.e., the temperature of the cryogenic container) is raised to a temperature compatible with introduction into the subject (generally from around room temperature to around body temperature, e.g., from about 20°C to about 37.6°C, inclusive), and the cells are introduced into a subject as discussed below.

V. $ALDH^{br}$ cells

In various embodiments of the present invention, at least the second cell population comprises ASPC that are $ALDH^{br}$. $ALDH^{br}$ cells express high levels of the enzyme aldehyde dehydrogenase and give low side scatter signals in flow cytometric
20 analysis. These cells are highly enriched in hematopoietic progenitor cells and comprise about 0.5% of the nucleated cells in freshly isolated human UCB. The various properties of $ALDH^{br}$ cell populations and methods of obtaining them are well known in art. See, for example, U.S. Patent No. 6,537,807; U.S. Patent No. 6,627,759; Storms *et al.* (1999) *Proc. Natl. Acad. Sci USA* 96:9118; PCT Publication No. WO2005/083061; Storms *et al.*
25 (2005) *Blood* 106(1):95-102; and, Hess *et al.* (2004) *Blood* 104(6):1648-55, each of which is herein incorporated by reference in their entirety.

VI. *Ex vivo* priming

Previous attempts to facilitate engraftment with *ex vivo* expanded cell populations
30 have failed. While not being bound to any particular mechanism of action, this may be because the cells were terminally differentiated in culture rendering them incapable of contributing to hematopoietic recovery in vivo. In one embodiment of the present invention, the second cell population of cells is primed, but not expanded, prior to administration to the subject. The *ex vivo* priming involves incubation of $ALDH^{br}$ UCB

in suitable culture medium containing one or more cytokines. Preferably, the cells are *ex vivo* primed for not more than 7 days, not more than 6 days, not more than 5 days, 4 days, 3 days, or not more than 2 days prior to introduction into the subject.

Many different cytokines useful in the methods of the present invention are those which have been used for *ex vivo* expansion of ASPC and are well known in the art. In one embodiment, the cells are cultured for 5 days prior to infusion with a cytokine cocktail consisting of stem cell factor (SCF), FLT-3, and interleukin 7 (IL-7) in a serum-free medium. The concentration of each cytokine can be determined empirically. In one embodiment, the concentration of each cytokine is about 5 ng/ml, about 10 ng/ml, about 15 ng/ml, about 20 ng/ml, 25 ng/ml, 30 ng/ml, 35 ng/ml, 40 ng/ml, 45 ng/ml, 50 ng/ml, 55 ng/ml, 60 ng/ml, 65 ng/ml, 70 ng/ml, 75 ng/ml, 80 ng/ml, 85 ng/ml, 90 ng/ml, 95 ng/ml, or about 100 ng/ml.

One of skill in the art will be able to determine a suitable growth medium for initial preparation of stem cells. Commonly used growth media for stem cells include, but are not limited to, Iscove's modified Dulbecco's Media (IMDM) media, SCGM™ (Cambrex, Baltimore, MD), DMEM, KO-DMEM, DMEM/F12, RPMI 1640 medium McCoy's 5A medium, minimum essential medium alpha medium (α -MEM), F-12K nutrient mixture medium (Kaighn's modification, F-12K), X-vivo 20, Stemline, CC100, H2000, Stemspan, MCDB 131 Medium, Basal Media Eagle (BME), Glasgow Minimum Essential Media, Modified Eagle Medium (MEM), Opti-MEM I Reduced Serum Media, Waymouth's MB 752/1 Media, Williams Media E, Medium NCTC-109, neuroplasma medium, BGJb Medium, Brinster's BMOC-3 Medium, CMRL Medium, CO.sub.2-Independent Medium, Leibovitz's L-15 Media, and the like.

Antibiotics, antifungals or other contamination preventive compounds can be added to the incubation medium, if desired. Exemplary compounds include but are not limited to penicillin, streptomycin, gentamycin, fungizone or others known in the art.

VII. Administration

The cell populations useful in the methods of the present invention have application in a variety of therapies and diagnostic regimens. They are preferably diluted in a suitable carrier such as buffered saline before administration to a subject. The cells may be administered in any physiologically acceptable vehicle. Cells are conventionally administered intravascularly by injection, catheter, or the like through a central line to

facilitate clinical management of a patient. This route of administration will deliver cells on the first pass circulation through the pulmonary vasculature. Usually, at least about 1×10^5 cells/kg and preferably about 1×10^6 cells/kg or more will be administered in the first cell population of cells, or in the combination of the first and second cell population.

5 See, for example, Sezer *et al.* (2000) *J. Clin. Oncol.* 18:3319 and Siena *et al.* (2000) *J. Clin. Oncol.* 18:1360. If desired, additional drugs such as 5-fluorouracil and/or growth factors may also be co-introduced. Suitable growth factors include, but are not limited to, cytokines such as IL-2, IL-3, IL-6, IL-11, G-CSF, M-CSF, GM-CSF, gamma-interferon, and erythropoietin. In some embodiments, the cell populations of the invention can be
10 administered in combination with other cell populations that support or enhance engraftment, by any means including but not limited to secretion of beneficial cytokines and/or presentation of cell surface proteins that are capable of delivering signals that induce stem cell growth, homing, or differentiation.

In some embodiments, first and/or second population of stem cells may be
15 conditioned by the removal of red blood cells and/or granulocytes after it has been frozen and thawed using standard methods.

The first and/or second population of stem cells may be administered to a subject in any pharmaceutically or medically acceptable manner, including by injection or transfusion. The cells or supplemented cell populations may contain, or be contained in
20 any pharmaceutically-acceptable carrier. For example, pharmaceutical compositions of the present invention may comprise a target cell population as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine;
25 antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the present invention are preferably formulated for intravenous administration. The first and/or second population of stem cells may be carried, stored, or transported in any pharmaceutically or medically
30 acceptable container, for example, a blood bag, transfer bag, plastic tube or vial.

A cell composition of the present invention should be introduced into a subject, preferably a human, in an amount sufficient to achieve tissue repair or regeneration, or to treat a desired disease or condition. Preferably, at least about 2.5×10^7 cells/kg, at least about 3.0×10^7 , at least about 3.5×10^7 , at least about 4.0×10^7 , at least about 4.5×10^7 ,

or at least about 5.0×10^7 cells/kg is used for any treatment, either in the first cell population, the second population, or a combination of the first and second population of stem cells. Where cord blood from several donors is used, the number of cord blood stem cells introduced into a subject may be higher. Where the first population of cells contains at least about 10^6 to about 10^8 nucleated cells per kg, the second population may contain significantly fewer cells. In various embodiments, the second population contains at least about 10^4 , or at least about 10^5 nucleated cells per kg. Thus, the methods of the invention may decrease the number of transplanted cells necessary for hematologic recovery. This method is particularly useful when the number of cells available for transplant is limited.

When "therapeutically effective amount" is indicated, the precise amount of the compositions of the present invention to be administered can be determined by an art worker with consideration of a subject's age, weight, tumor size, extent of infection or metastasis, and condition of the subject. The cells can be administered by using infusion or injection techniques that are commonly known in the art.

VIII. Adjuvant therapy

In accordance with the use of first and second population of stem cells in the methods of the invention, one may also treat the host to reduce immunological rejection of the donor cells, such as those described in U.S. Pat. No. 5,800,539, issued Sep. 1, 1998; and U.S. Pat. No. 5,806,529, issued Sep. 15, 1998, both of which are incorporated herein by reference.

In certain embodiments of the present invention, the cells of the present invention are administered to a subject following treatment with an agent such as myeloablative (high dose) chemotherapy, chemotherapy, radiation, immunosuppressive agents, such as antithymocyte globulin (ATG), busulfan, IVIG, melphalan, methylprednisolone, cyclosporin, azathioprine, methotrexate, mycophenylate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludaribine, cyclosporin, FK506, rapamycin, mycophenylic acid, steroids, FR901228, cytokines, and localized or total body irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin). (Liu et al., Cell 66:807-815, 1991; Henderson et al., Immun. 73:316-321, 1991; Bierer et al., Curr. Opin. Immun. 5:763-773, 1993; Isoniemi (supra)). In a further embodiment, the cell compositions of the present invention are administered to a subject in conjunction with

(e.g. before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another embodiment, the cell compositions of the present invention are administered following B-cell ablative therapy such as agents that react with CD20, e.g. Rituxan. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by stem cell transplantation. Following the transplant, subjects receive an infusion of the two cell populations described herein.

The dosage of the above treatments to be administered to a subject will vary with the precise nature of the condition being treated and the recipient of the treatment. The scaling of dosages for human administration can be performed according to art-accepted practices.

IX. Monitoring therapeutic response

Methods for monitoring therapeutic response in subjects include assessment of one or more of overall and event-free survival, , platelet engraftment, ANC engraftment, relapse of disease, or the like, in a subject. The response to treatment can be compared to an appropriate control. Methods for monitoring these responses are well known in the art and exemplified herein.

For the purposes of the present invention, a “subject” refers to an individual that has been administered the cell preparations of the invention. The subject can be a human, a non-human primate, a laboratory animal, or the like, but preferably is a human. A “control” can include an individual (or group of individuals) that is (are) untreated, sham treated (e.g., the individual is treated with a first and second cell population in which one or both populations do not contain the cell preparations described herein), treated with a similar or distinct method for improving engraftment and/or improving therapeutic response to stem cell transplantation, or treated with a cell preparation that is different from the cell populations described herein, depending on the nature of the observation. For example, if one wishes to compare the therapeutic response of a subject that has been treated with a second cell population that has been *ex vivo* cytokine primed, an appropriate control may include a subject that has been treated with a second cell population that has not been primed, or may include the therapeutic response of a subject whose second cell population has been cultured without using a priming agent. Alternatively, controls can be historical controls. For example, the response of the

subject to the methods of the invention can be compared to the response seen in previously studied populations of subjects undergoing similar or distinct procedures for modulating engraftment and/or improving therapeutic response to stem cell transplantation.

5 In some embodiments, the methods of the present invention result in a decrease of incidence and/or severity of grade III and/or grade IV acute graft versus host disease (GvHD), in part by eliminating T cell populations. This elimination from the stem cell population of the invention can be expected to reduce the incidence and severity of GvHD in recipients of allogeneic transplants. See, for example, Ho and Soiffer (2001) *Blood*
10 98:3192. GvHD occurs when donor T-cells react against antigens on normal host cells causing target organ damage, which often leads to death. The principal target organs of GvHD are the immune system, skin, liver and intestine.

There are two kinds of GvHD: acute and chronic. Acute GvHD appears within the first three months following transplantation. Signs of acute GvHD include a reddish skin
15 rash on the hands and feet that may spread and become more severe, with peeling or blistering skin. GvHD is ranked based on its severity: stage (or grade) 1 is mild, stage (or grade) 4 is severe. Chronic GvHD develops three months or later following transplantation. The symptoms of chronic GvHD are similar to those of acute GvHD, but in addition, chronic GvHD may also affect the mucous glands in the eyes, salivary glands
20 in the mouth, and glands that lubricate the stomach lining and intestines.

Following administration of the cell populations described herein, the subject may be monitored for levels of malignant cells, i.e., for evidence of minimal residual disease. Such monitoring may comprise subject follow-up for clinical signs of relapse. The monitoring may also include, where appropriate, various molecular or cellular assays to
25 detect or quantify any residual malignant cells. For example, in cases of sex-mismatched donors and recipients, residual host-derived cells may be detected through use of appropriate sex markers such as Y chromosome-specific nucleic acid primers or probes. In cases of single HLA locus mismatches between donors and recipients, residual host cells may be documented by polymerase chain reaction (PCR) analysis of Class I or Class
30 II loci that differ between the donor and recipient. Alternatively, appropriate molecular markers specific for tumor cells can be employed. For example, nucleic acid primers and/or probes specific for the bcr/abl translocation in chronic myelogenous leukemia, for other oncogenes active in various tumors, for inactivated tumor suppressor genes, other tumor-specific genes, or any other assay reagents known to be specific for tumor cells,

may be employed. Any of these or functionally comparable procedures may be used to monitor the subject for evidence of residual malignant cells. In one embodiment, the methods of the present invention result in at least about a 10%, at least about a 15%, at least about a 20%, about a 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or at least about a 100% decrease in the presence of malignant cells when compared to a control.

Treatment of a subject according to the methods of the present invention may be considered efficacious if the disease, disorder or condition is measurably improved in any way. Such improvement may be shown by a number of indicators. Measurable indicators include, for example, detectable changes in a physiological condition or set of physiological conditions associated with a particular disease, disorder or condition (including, but not limited to, blood pressure, heart rate, respiratory rate, counts of various blood cell types, levels in the blood of certain proteins, carbohydrates, lipids or cytokines or modulated expression of genetic markers associated with the disease, disorder or condition). Treatment of an individual with the stem cells or supplemented cell populations of the invention would be considered effective if any one of such indicators responds to such treatment by changing to a value that is within, or closer to, the normal value. The normal value may be established by normal ranges that are known in the art for various indicators, or by comparison to such values in a control. In medical science, the efficacy of a treatment is also often characterized in terms of an individual's impressions and subjective feeling of the individual's state of health. Improvement therefore may also be characterized by subjective indicators, such as the individual's subjective feeling of improvement, increased well-being, increased state of health, improved level of energy, or the like, after administration of the cell populations of the invention. In one embodiment, the methods of the present invention result in at least about a 30%, at least about a 35%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, about 125%, about 150%, about 175%, about 200%, about 250%, at least about a 300%, or greater improvement in one or more of the clinical indicators described above when compared to a control.

The primary measure of hematologic recovery is neutrophil count. Neutrophils usually constitute about 45 to 75% of all white blood cells in the bloodstream. When the neutrophil count falls below 1,000 cells per microliter of blood, the risk of infection

increases somewhat; when it falls below 500 cells per microliter, the risk of infection increases greatly. Without the key defense provided by neutrophils, controlling infections is problematic and subjects are at risk of dying from an infection. Accordingly, in clinical settings, such as transplant settings, the sooner neutrophil counts recover, the sooner a subject can be released from the hospital. Accordingly, any decrease in time that it takes to achieve clinically relevant levels of neutrophils is beneficial to the subject and contemplated herein as acceleration of hematologic recovery. For the purposes of the present invention, neutrophil engraftment is defined as an absolute neutrophil count (ANC) of at least 500 neutrophils/ μ l. The neutrophil count may be reported as a date that an individual subject (or an average of multiple subjects) reaches the ANC threshold, or a percentage of the subjects having an ANC of 500 neutrophils/ μ l by a particular day post-transplant, usually around day 42, or the probability that an individual will reach a certain threshold by a certain date. In one embodiment, the methods of the present invention result in neutrophil engraftment on or before day 10, day 11, day 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, or on or before day 48. In another embodiment, the day that patients achieve a benchmark ANC count deemed to be normal will be accelerated by 5 days, 6 to 10 days, 11-20 days, or greater than 20 days relative to a control group of patients.

Hematologic recovery can also be measured by a clinically relevant recovery of platelets (as would be recognized by the skilled artisan, there are normally between 150,000-450,000 platelets in each microliter of blood). Thus, any increase in the rapidity of a clinically relevant recovery of platelets is advantageous and contemplated herein. For the purposes of the present invention, platelet engraftment is defined as maintenance of platelet counts of at least 50,000 platelets/ μ l of blood without transfusion support. The platelet count may be reported as a date that an individual subject (or an average of multiple subjects) reaches the platelet count threshold, or as a percentage of the subjects having (or probability of a subject reaching) a platelet count of at least 50,000 platelets/ μ l of blood by a particular day post-transplant, usually around day 180. In one embodiment, the methods of the present invention result in platelet engraftment on or before day 50, day 55, day 60, 65, 70, 75, 80, 85, 90, 95, or on or before day 100. In another embodiment, the day that patients achieve a benchmark platelet count deemed to be

normal will be accelerated by 5 days, 6 to 10 days, 11-20 days, or greater than 20 days relative to a control group of patients.

In certain embodiments, rapidity in T cell recovery is also an indicator of accelerated hematologic recovery. An indicator of T cell recovery can include response to PHA-induced proliferation and/or an increase in the number of CD4+ cells in the subject. The CD4+ counts may be reported as a date that an individual subject (or an average of multiple subjects) reaches a CD4+ count threshold, or as a percentage of the subjects having (or the probability of subject reaching) a threshold CD4+ count by a particular benchmark day post-transplant, usually around day 100. In one embodiment, the methods of the present invention result in T cell counts at day 100 that are at least about 25 to 100% or greater than counts in patients in a control population. In another embodiment, the post-transplant day that a patient achieves a benchmark CD4 count is about 10 to about 20, about 20 to about 30, about 30 to about 40, about 40 to about 50, or greater than 50 days earlier than the day that patients in a control group achieve the same benchmark CD4 count.

A therapeutic response can also be measured in terms of overall and/or event free survival. Event free survival (EFS) is defined as the time from transplantation to the day of the first event. Events are defined as graft failure, autologous reconstitution, relapse, or death. Relapse in leukemic subjects is determined by standard criteria. Tertiary end points include description of the incidence of acute GvHD, and other measures of nonrelapse mortality. GvHD is scored according to standard criteria (Przepiorka et al. (1995) *Bone Marrow Transplant*. 15: 825-828). In one embodiment, the methods of the present invention result in overall and/or event-free survival that is at least about 30%, at least about 35%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 125%, 150%, 175%, 200%, 250%, at least about 300%, or greater % improved over controls (e.g., fewer or no incidences of events reported (particularly grade III and/or grade IV acute GvHD), increased number of days of survival, and/or higher numbers of patients surviving to a certain date post-transplant when compared to a control population).

Another global measure of therapeutic response is overall survival at 180 days. In this metric, survival in the group of patients transplanted according to the present invention is compared to overall survival in a control group treated by conventional methods. In one embodiment of the invention, patients show an improved overall survival of at least about 5%, of at least about 6-10%, of at least about 11-15%, of at least about 16-20%, or of great than 20% compared to control patients.

The following examples are offered by way of illustration and not by way of limitation.

5

EXPERIMENTAL

Example 1. Immune Reconstitution after Unrelated Mismatched UCB Transplantation:

Immune reconstitution has been evaluated in approximately 100 survivors of UCB transplantation that have been followed for a median of 650 days (range 121-2450 days).
10 The results of this study can be found in Klein et al. (2001) *Biol Blood and Marrow Trans* 7:454-466. Briefly, functional and immunophenotypic parameters were assayed in engrafted patient's peripheral blood at 3, 6, 9, 12, 24, and 36 months post transplant. Patients were generally maintained on methyprednisolone for the first three months post transplant and cyclosporine for the first year post transplant. Immunizations were
15 reinstituted in the second and third years post transplant. All surviving patients without active chronic GvHD received the full complement of killed and live vaccines per the usual CDC recommendations. Infants and toddlers <2 years of age recovered T-cell immune function as measured by CD4 counts and PHA responses by 6 months post transplant. Children between the ages of 2 – 12 years recovered similar function by 9-12
20 months post transplant. In contrast, teenagers and adults recovered immune function by 3 years post-transplant. It appears that the host thymus contributes to immune reconstitute from the UCB graft. The younger the patient and the healthier the thymus (e.g. no exposure to pre-transplant irradiation), the quicker the thymic recovers and contributes to immune reconstitution from the graft. Children normalized by 1 year post transplant,
25 while adults approached the lower limit of normal for age by 3 years post transplant. In the interim, adults reconstituted T-cells by peripheral mechanisms. Those patients with earlier immune reconstitution faired better with transplant overall. They were less likely to develop an opportunistic infection in the first 2-4 months post transplant. The patients in this category had superior survival. Percent CD4 cells was the best predictor of lack of
30 opportunistic infection ($p = <0.001$).

Example 2. Clinical Results of UCB Transplantation in Pediatric Patients with Inborn Errors of Metabolism

Recent results from the Cord Blood transplantation Study (COBLT), a multi-institutional, prospective NIH-sponsored trial of unrelated donor cord blood transplantation have further advanced the field of UCBT. See, Kurtzberg *et al.* (2005) *Biology of Blood and Marrow Transplantation* 11(2):2 (abst 6); Kurtzberg *et al.* (2005) *Biology of Blood and Marrow Transplantation* 11(2):82(Abst 242).

A different strata of the COBLT study evaluated the efficacy of cord blood transplantation in 69 children with inborn errors of metabolism, augmenting prior and pending reports results of UCBT in babies with Infantile Krabbe Disease and Hurler Syndrome (MPS I). A common protocol was used for the preparative regimen (busulfan, cyclophosphamide, ATG) and GvHD prophylaxis (cyclosporine, steroids). Patients with MPS 1-V (n=36, 20 previously reported), globoid cell leukodystrophy (Krabbe Disease, n=16), adrenoleukodystrophy (n=8), metachromatic leukodystrophy (n=6) and Tay Sachs Disease (n=3) with a median age of 1.8 years (range 0.1-11.7 years) and a median weight of 12.3kg (range 3.9-42.3kg) were transplanted with partially HLA-mismatched unrelated donor cord blood delivering a median of 8.7×10^7 nucleated cells/kg (range 2.8-38.8 cells/kg) selected from COBLT (83%) or other (17%) banks. CBUs were screened for enzyme activity to prevent use of carrier donors. Sixty-four percent of patients were male and 77% were Caucasian. Nearly half the patients (48%) received a UCB units matching at 4/6 HLA loci as measured by low resolution typing at HLA Class I A&B and high resolution typing at HLA Class II DRB1.

The cumulative incidence of neutrophil engraftment (ANC 500/uL with 90% donor chimerism by day 100) was 78%, occurring in a median of 26 days. The cumulative incidence of acute Grades II-IV GvDH was 46%. The probability of survival at 180 days and 1 year was 80 and 72%, respectively. Levels of HLA disparity between recipient and donor did not influence engraftment, GvHD or overall survival. The surviving patients with MPS, TSD, GLD, and MLD all stabilized and/or gained skills post transplant. Three of 8 patients with ALD, all of whom had mild to moderate clinical symptoms at the time of referral for transplant, experienced disease progression with neurologic deterioration before stabilization. Outcomes in babies with the severe phenotype of Hurler Syndrome (Kurtzberg, 2005, *supra* and Dexter *et al.* (1977) *J Cell Physiol* 91:335-344) and newborns with Krabbe Disease (Gartner *et al.* (1980) Gartner

Proc Natl Acad Sci 77:4756-4759) transplanted before the onset of symptoms were unprecedented with the vast majority of patients having normal intelligence quotients for age. The younger the age at transplant and the earlier in the course of the disease, the better the overall outcome. Therefore, it is clear that cord blood transplantation offers a rapidly available donor source for early treatment of infants, toddlers and children with inborn errors of metabolism

Example 3. Prepurification steps to enrich for ALDH^{br} UCB cells

The cord blood unit selected for transplantation was stored in a 2 compartment cryopreservation bag (20%/80% split) in a total of 25ml of cells, hespan and 10% DMSO. On day -5 before transplant, the 20% (5ml) fraction was removed from liquid nitrogen (procedure 5D.160.01), and thawed in a 37 degree C waterbath to a slushy consistency. Dextran/Albumin was added to dilute to 4x the initial volume, the cells were washed, pelleted and resuspended in ALDESORT® assay buffer/100U/ml DNase I (Aldagen, Inc., Durham, NC). Red blood cell to white blood cell ration was adjusted to $<1 \times 10^8$ cells/ml and the cells were lineage depleted with EASYSEP® (StemCell Technologies) anti-glycophorin A and CD14 cocktails to label cells. The labeled cells were mixed with EASYSEP® magnetic nanoparticles and incubated at room temperature for 10 minutes. The sample was then exposed to the EASYSEP® magnetic which will remove lineage positive cells. The residual lineage depleted cells were gently aspirated into a conical tube. RBC:WBC ratio was checked and must have been $<1:10$. If it was higher, the EASYSEP® depletion was repeated.

Example 4. Isolation of ALDH^{br} UCB cells by high speed FACS sorting

The lineage depleted cells were stained with activated ALDESORT® reagent and incubated at 37 degrees C for 15 minutes. The reaction was stopped, controls were prepared and the ALDH^{br} cells were isolated by high speed flow sorting on the FACS Aria sorter (BD Biosciences). Methods for isolating ALDH^{br} cells are more fully described in Storms *et al.*, 1999, *supra* and PCT Publication No. WO 2005/083061, both of which are herein incorporated by reference in their entirety. The cells may be frozen, infused, or further primed as described in Example 5.

Example 5. Thawing, sorting, priming, and infusion of the ALDH^{br} cells

The UCB cells were thawed, ALDH^{br} sorted and cytokine primed 5 days prior to the scheduled conventional UCB transplant (UCBT). Briefly, the 20% fraction of the UCB unit was removed from storage, thawed in a 37°C degree water-bath, mixed with dextran and albumin and washed. The resulting cell pellet was resuspended in EASYSEP® medium (Stem Cell Technologies) to remove lineage positive cells. The residual lineage negative cells were RBC cell depleted to achieve a WBC:RBC ratio of <1:10. This cell population was sorted on a FACSaria (Becton Dickenson) to isolate a purified population of ALDH^{br} cells. The ALDH^{br} cells was placed in culture with a cytokine cocktail consisting of SCF 50ng/ml, FLT-3 10ng/ml and IL-7 10ng/ml in serum-free medium (Cellgenix SCGM) and incubated in 5% CO₂ at 37 degrees C in diffusion exchange bags (American Fluoseal) for 5 days. At the completion of culture, ALDH^{br} primed cells were transferred to a standard transfer pack with an attached bag of normal saline for infusion.

On day 0, transplant day, approximately 4 hours after infusion of the conventional UCB graft, the cytokine primed ALDH^{br} UCB cells were harvested, counted, checked for viability and gram stain, connected to the infusion set and transported to the bone marrow transplant unit for infusion.

Example 6. UCB Thawing and Infusion for the conventional, unmanipulated graft (first cell population)

Bags of UCB were thawed in the laboratory using sterile technique under a hood. The UCB was thawed in a 37°C waterbath, and diluted by 1:1 volume using a 5% albumin /dextran solution [albumin 25% (12.5 gms/50 ml) 25 gms in 500 ml dextran] to preserve cell viability. The 5% albumin /dextran solution was added slowly to the thawed UCB using transfer bags with stopcocks and mixed gently. The thawed and diluted UCB was next weighed and centrifuged (2000 rpm x 20 min at 4° C). Specimens were obtained for cell count and viability, culture, clonogenic assays, and phenotype. Supernatant containing DMSO and the albumin/ dextran solution was removed, and the UCB pellet resuspended again by a 1:1 volume using a 5% albumin/dextran solution. The UCB was labeled with patient identification information and transferred to the bedside for infusion. The UCB was infused via the patient's central venous catheter at a rate of 1-3 ml/min. UCB was infused without an in-line filter and was not irradiated. If the patient developed chest tightness or other symptoms, a brief rest (1-2 minutes) was

allowed before proceeding with the remainder of the infusion. If a large volume of UCB (>15 ml/kg) was to be infused, half the UCB may have been infused, followed by a 30 minute rest period, and then infusion of the remainder of the UCB. Vital signs were taken every 15 minutes until 2 hours after completion of the infusion. Hydration (2.5-3.0 ml/kg/hr) was maintained for 12 hours after UCB infusion was completed. Furosemide (0.5-1.0 mg/kg/dose) was given if volume overload or decreased urine output occurs.

Example 7. Conditioning of Patients with Malignant Conditions

Standard cytoreduction for patients with ALL undergoing allogeneic BMT includes cyclophosphamide (100-200 mg/kg) and total body irradiation (TBI, 1,000-1440 cGy). With these regimens, event-free survival rates can be achieved in 20-45% of children and 20% of adults with ALL in 2nd remission, and up to 60% of patients with ANLL undergoing matched-related allogeneic BMT. With subsequent remissions, event-free survival decreases with only 8% of patients cured when transplanted in relapse. ATG was used for additional immunosuppressive therapy; methylprednisolone was substituted if patients could not tolerate ATG.

The rates of engraftment, GvHD, relapse and survival from the COBLT study (Klein *et al.* 2001, *supra*) were used as historical controls to benchmark the success of the transplant.

Example 8. Conditioning of Patients with Non-Malignant Conditions

Standard cytoreduction for patients with non-malignant conditions undergoing allogeneic BMT includes busulfan 16 mg/kg over 4 days (adjusted for pediatric patients to dosing per m² and followed with targeted levels with first dose PK), cyclophosphamide 200 mg/kg over 4 days and ATG 90 mg/kg over 3 days. Engraftment rates with unrelated donor umbilical cord blood using this regimen ranges between 80-90%. TBI was avoided to minimize late adverse events such as growth retardation, endocrine failure, cognitive deficits, chronic lung disease or cardiomyopathy.

Example 9. Ex Vivo expansion of ALDH^{br} UCB cells after priming

To assess the capacity of ALDH^{br} cytokine primed UCB cells, 5 day cultures were harvested and incubated for 2 more weeks in expansion medium. TNC, viability, clonal hematopoietic progenitor cell growth and expansion of ALDH^{br}, lineage negative cells were scored. In 7 separate experiments, the mean expansion of total nucleated cells was

10.74 $\pm$ 10.62 fold. Individual results are shown in Table 1 below. On morphologic examination, approximately 50% of the expanded population had the appearance of blast cells.

5 Table 1. Fold Expansion at Day 12. Samples cultured in IMDM/10% FCS/10% HS, sodium pyruvate, non-essential amino acids, 50 ng/ml SCF, 10 ng/ml IL-7, 10 ng/ml FLT3-L, 10 ng/ml TPO, and 10 ng/ml GM-CSF from day 5 to day 12.

Sample ID	1588	1709	1552	1554	1556	1573	1575	Average	Std Dev
Fold Expansion	7.04	3.99	8.15	0.80	32.89	7.92	14.40	10.74	10.62

10 Example 10. Evaluation of Engraftment

Peripheral blood samples were tested on or about days + 30, 60 and 100 for chimerism. A bone marrow aspirate and biopsy for cellularity and donor chimerism was performed between days 41-44 if the patient had not demonstrated neutrophil recovery by this time. Platelet counts, ANC, and various other clinical indicators of successful
15 engraftment were evaluated as known in the art. The results for primed and unprimed samples were combined for statistical evaluation of engraftment. The rate of neutrophil engraftment is shown in Figures 1 and 2. The rate of platelet engraftment is shown in Figures 3 and 4.

Many modifications and other embodiments of the inventions set forth herein will
20 come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims and list of embodiments disclosed
25 herein. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same
30 extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

THAT WHICH IS CLAIMED:

1. A method of reconstituting blood tissue in a subject in need thereof, said
5 method comprising introducing into said subject a first and a second population of cells
derived from umbilical cord blood, wherein said second population of cells is introduced
between 2 and 24 hours after the first population of cells, wherein at least the second
population is an enriched ALDH^{br} stem cell population.

10 2. The method of claim 1, wherein said second population of cells is
introduced about 4 hours after the first population.

3. The method of claim 1, wherein the first and second populations of cells
are derived from the same cord blood unit or donor.

15 4. The method of claim 1, wherein the first and second populations of cells
are derived from different donors.

20 5. The method of claim 1, wherein said subject is in need of hematopoietic
reconstitution following bone marrow ablation.

6. A method of restoring hematologic function following myeloablative
treatment in a subject having cancer, said method comprising introducing into said
subject a first and a second population of cells, wherein said second population of cells is
25 introduced between 2 and 24 hours after the first population of cells, wherein at least the
second population is an enriched ALDH^{br} stem cell population.

7. The method of claim 6, wherein said subject is in need of treatment for
sequelae related to cancer therapy.

30 8. A method for accelerating hematopoietic recovery in a subject following
myeloablation comprising introducing into said subject a first and a second population of
cells, wherein said second population of cells is introduced between 2 and 24 hours after

the first population of cells, and wherein at least the second population is an ALDH^{br} stem cell population.

9. The method of claim 8, wherein the time to neutrophil engraftment in the subject is shortened compared to the time to neutrophil engraftment in a control subject.

10. The method of claim 8, wherein the time to platelet engraftment is shortened compared to the time to platelet engraftment in a control subject.

11. A method of restoring hematologic function following myeloablative treatment in a subject having a genetic disorder comprising introducing into said subject a first and a second population of cells, wherein said second population of cells is introduced between 2 and 24 hours after the first population of cells, wherein at least the second population is an enriched ALDH^{br} stem cell population.

12. A method of restoring bone marrow stem or progenitor cell activity following myeloablative treatment in a subject having cancer comprising introducing into said subject a first and a second population of cells, wherein said second population of cells is introduced between 2 and 24 hours after the first population of cells, wherein at least the second population is an enriched ALDH^{br} stem cell population.

13. A method of restoring bone marrow stem or progenitor cell activity following myeloablative treatment in a subject having a genetic disorder comprising introducing into said subject a first and a second population of cells, wherein said second population of cells is introduced between 2 and 24 hours after the first population of cells, wherein at least the second population is an enriched ALDH^{br} stem cell population.

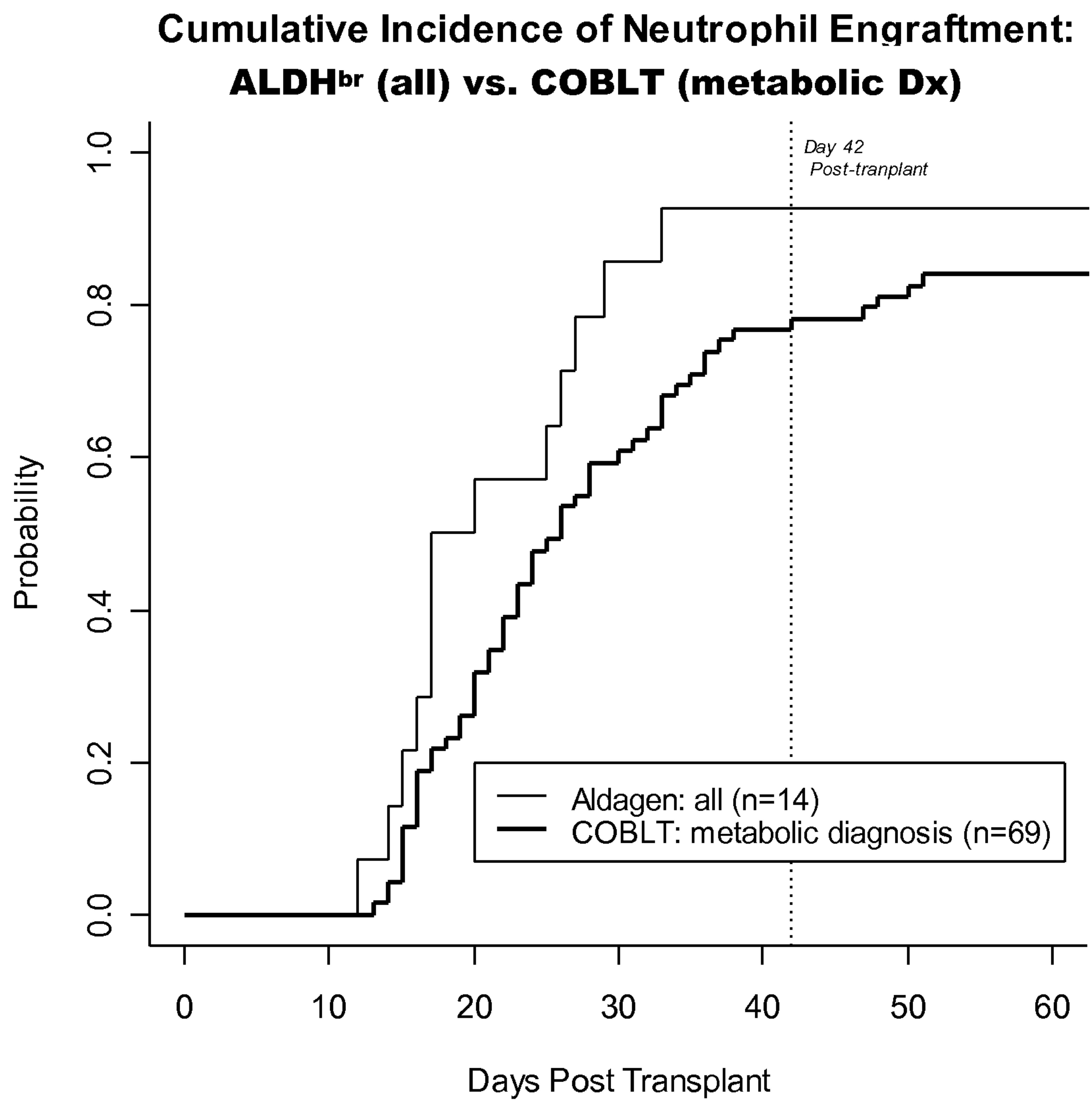


FIG. 1
1/4

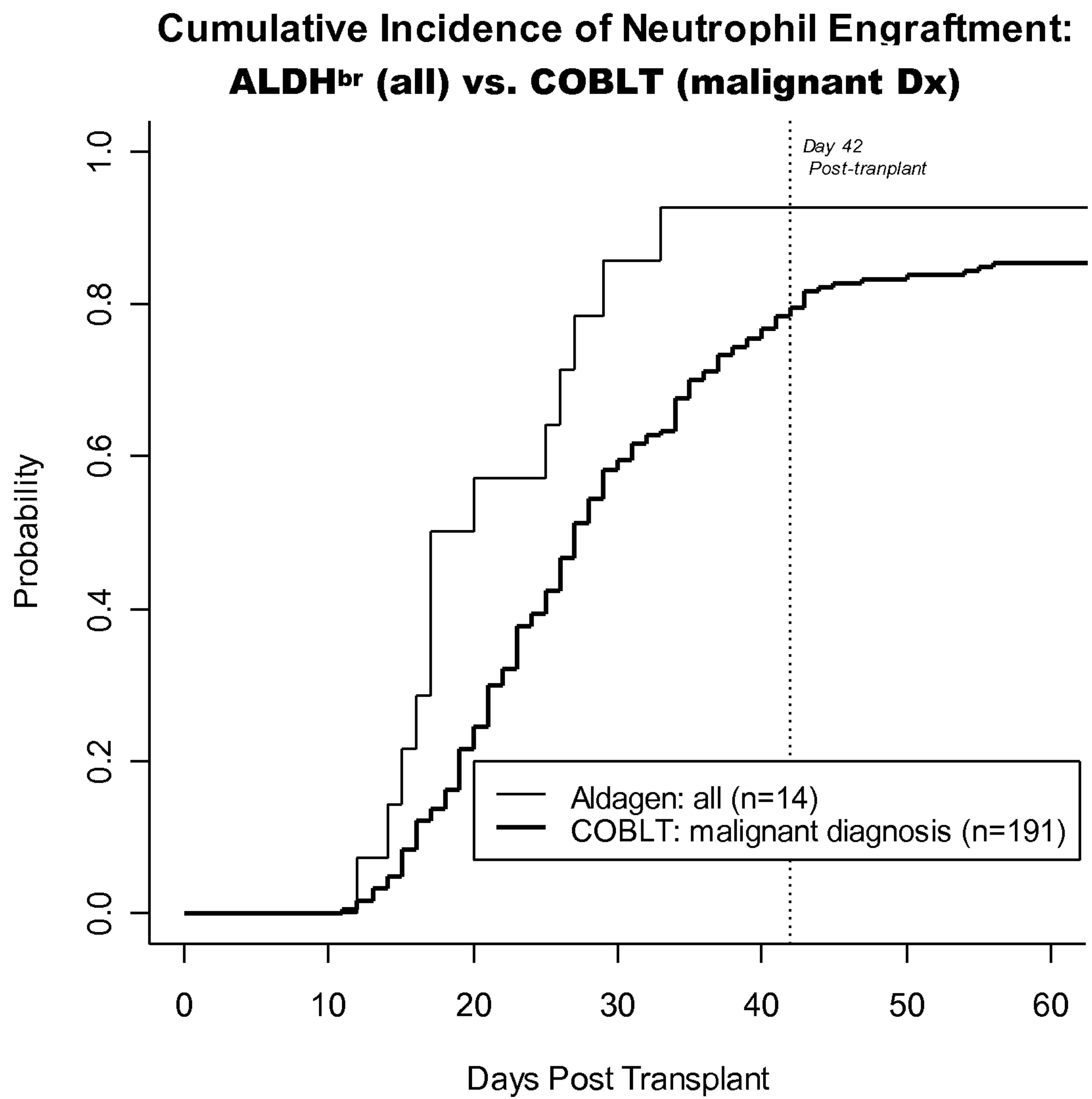


FIG. 2
2/4

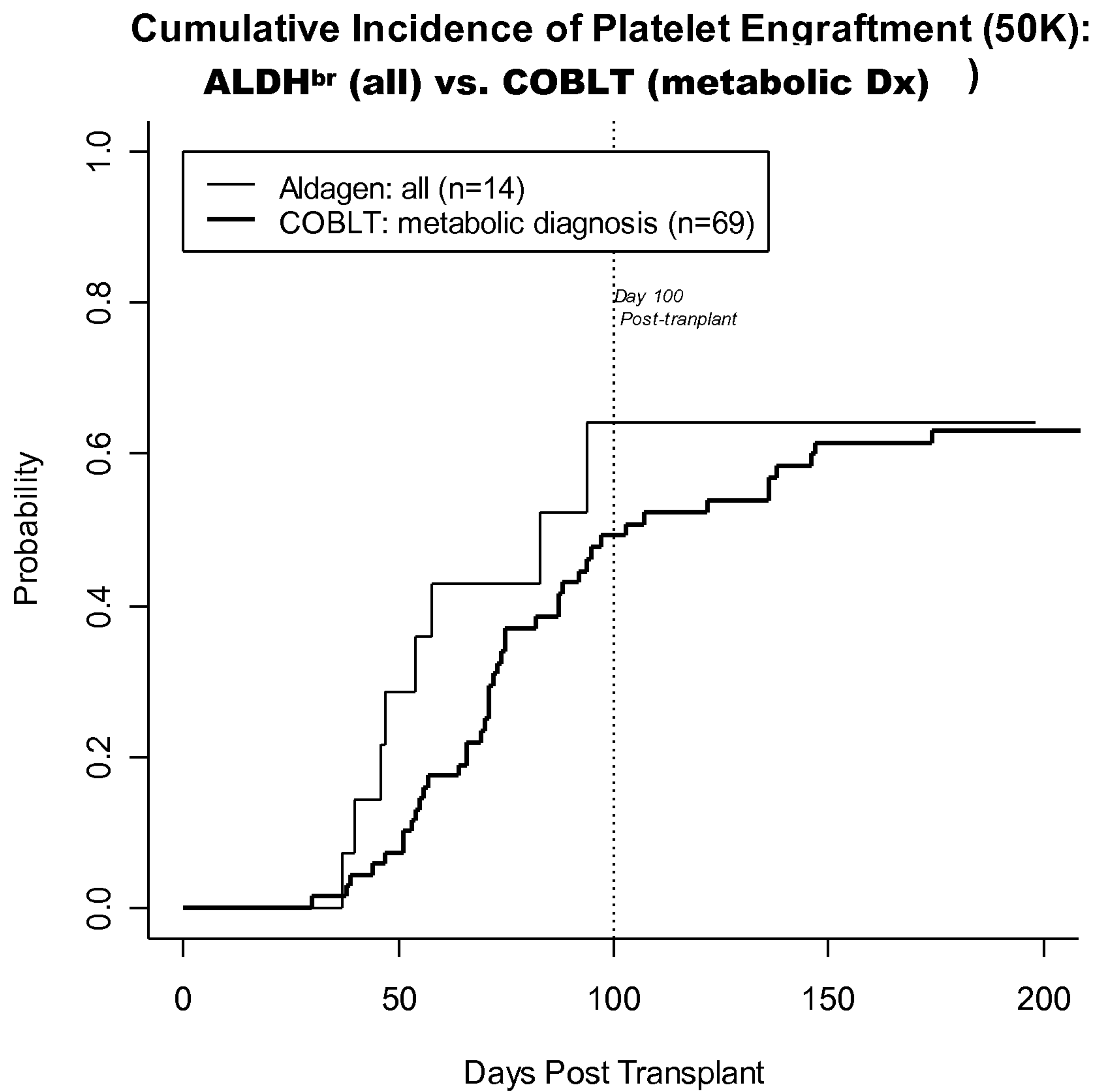


FIG. 3
3/4

**Cumulative Incidence of Platelet Engraftment (50K):
ALDH^{br} (all) vs. COBLT (malignant Dx)**

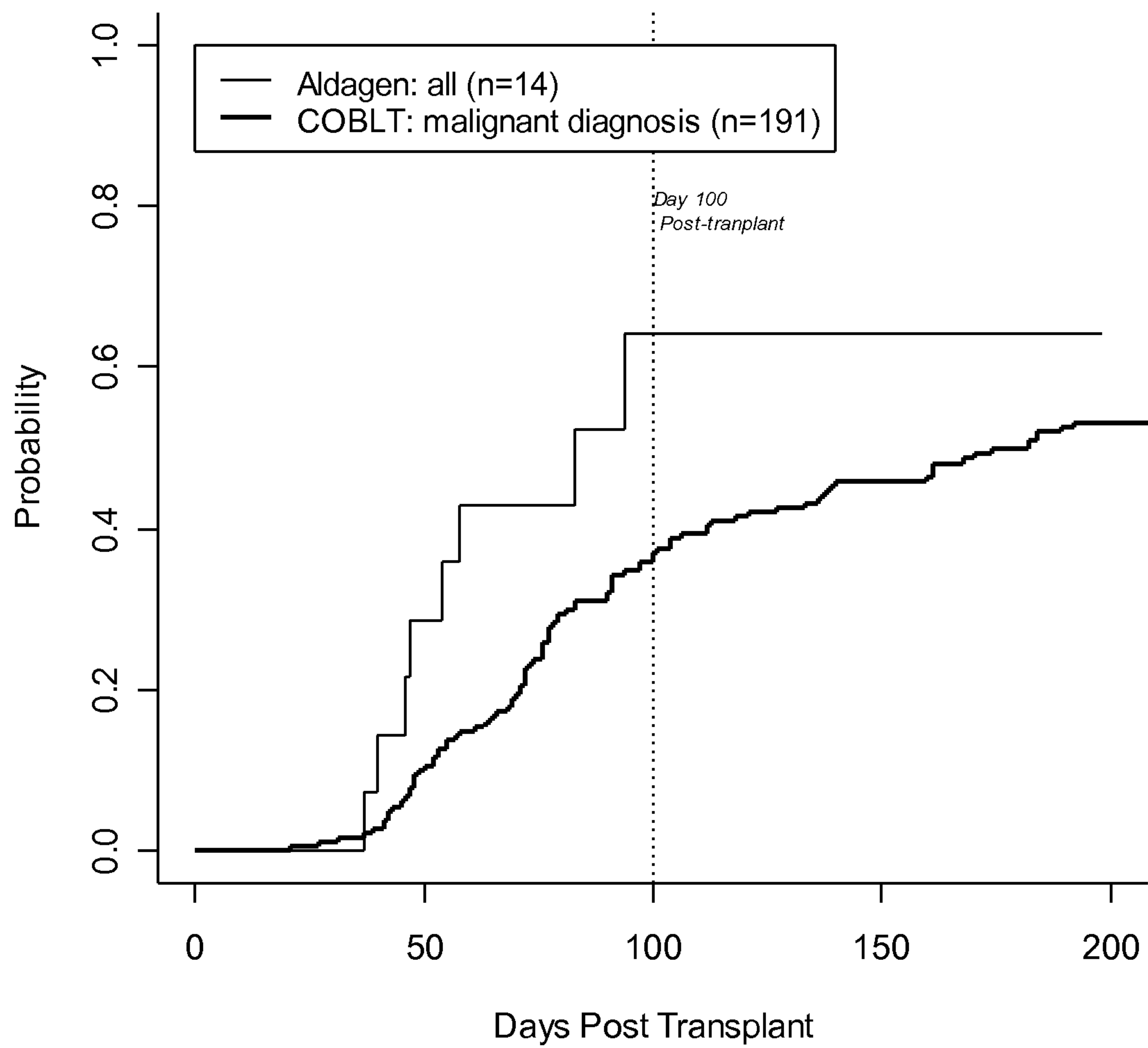


FIG. 4
4/4