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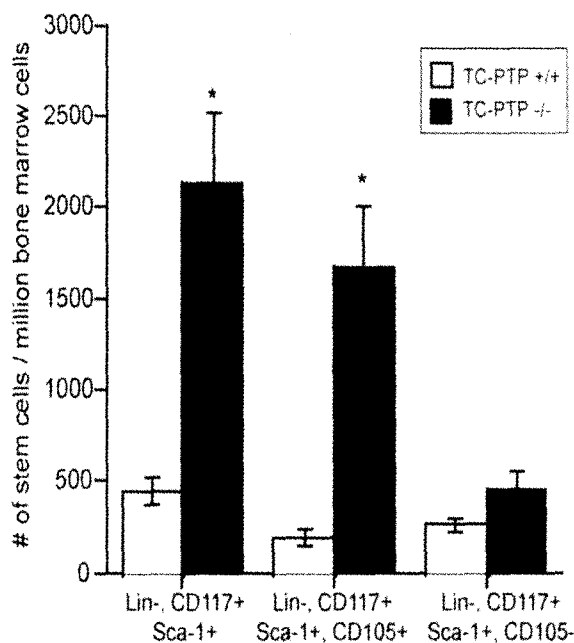
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(54) Title: AUGMENTING STEM CELL POPULATIONS BY MODULATING T-CELL PROTEIN TYROSINE PHOSPHATASE (TC-PTP)



(57) Abstract: A method of increasing stem cell expansion and isolating stem cells is disclosed. The method of expanding stem cells comprises incubating cells with an agent that inhibits T-cell protein tyrosine phosphatase (TC-PTP).

WO 2008/043181 A1

- 1 -

Title: AUGMENTING STEM CELL POPULATIONS BY MODULATING T-CELL PROTEIN TYROSINE PHOSPHATASE (TC-PTP)

FIELD OF THE INVENTION

[0001] The present invention relates to methods of propagating stem cells and expanding stem cell populations including endothelial progenitor cells. The invention further provides methods for using stem cells and
5 endothelial progenitor cells for various therapeutic applications including revascularization of ischemic tissues.

BACKGROUND

[0002] Adult bone marrow contains stem cells able to reconstitute the entire hematopoietic and vascular systems (Figure 1). The ordered
10 progression of stem cells and more differentiated precursor cells through successive developmental stages must be tightly controlled. Tyrosine phosphorylation of receptors, adaptors and structural proteins plays a key role in regulating the development of hematopoietic and endothelial cells. To date, attention has focused mainly on protein tyrosine kinases (PTK), which initiate
15 and modulate these signaling events. The potential contribution of protein tyrosine phosphatases (PTP), the counterparts of PTK, has not been investigated.

A role for TC-PTP in hematopoietic development

[0003] The T-cell protein tyrosine phosphatase (TC-PTP) is an
20 intracellular enzyme encoded by *Ptpn2*. It is ubiquitously expressed in embryonic and adult cells, with highest levels in hematopoietic tissues (reviewed in Bourdeau et al., 2005).

TC-PTP gene

[0004] Human TC-PTP was identified by the screening of a human
25 peripheral T-cell cDNA library with labeled oligonucleotides derived from the catalytic domain of the PTP1B protein tyrosine phosphatase. The cDNA sequence of human TC-PTP contains a full open reading frame of 1305 base pairs (bp), and a 978 bp 3'-untranslated region (Cool et al. Proc Natl Acad Sci

- 2 -

U S A. 1989 Jul;86(14)). Work describing the mapping of human TC-PTP to chromosome 18p11.2-p11.3 also identified two TC-PTP pseudogenes that were localized on chromosomes 1 and 13. Although these pseudogenes have considerable sequence similarity with TC-PTP, neither of these pseudogenes
5 could be translated in a single reading frame in order to generate a complete TC-PTP protein or any other known PTPs (Johnson et al. Genomics. 1993 Jun;16(3):619-29).

[0005] Subsequently, the mouse homologue of TC-PTP, termed PTP-2 or MPTP was described (Miyasaka and Li Mol Cell Biochem. 1992 Dec
10 2;118(1):91-8; Mosinger et al. Proc Natl Acad Sci U S A. 1992 Jan 15;89(2):499-503). The mouse gene is 88.8% identical to human TC-PTP at the nucleotide level, and maps to chromosome 18, a region of synteny with the human TC-PTP locus. The murine cDNA sequence comprises 1570 bp and contains a 5'-untranslated region of 61 bp, a single full open reading
15 frame of 1146 bp and a 3'-untranslated region that includes a polyadenylation signal located 90 bp upstream of the polyadenylation site (Mosinger et al. Proc Natl Acad Sci U S A. 1992 Jan 15;89(2):499-503).

TC-PTP protein

[0006] The cloning of the cDNA for human TC-PTP identified an
20 intracellular protein comprising 418 amino acid (aa) residues (Cool et al. Proc Natl Acad Sci U S A. 1989 Jul;86(14):5257-61). Sequence comparison with the murine TC-PTP cDNA revealed 95% identity at the protein level, with the highest homology in the single catalytic domain (Mosinger et al. Proc Natl Acad Sci U S A. 1992 Jan 15;89(2):499-503). However, the human and
25 murine TC-PTP open reading frames differed markedly in their 3' ends. This observation led to the recognition of a splice donor site (ACGT) in the human TC-PTP gene at the position where the sequence diverges from that of its murine counterpart. This splicing event generated two distinct mRNAs encoding proteins termed TC-PTPa and TC-PTPb of predicted size of 45 kDa
30 and 48.5 kDa respectively (Champion-Arnaud et al. Oncogene 1991 Jul;6(7):1203-9)). TC-PTPa mRNA contains a unique exon originating from

- 3 -

the 3' end of the TC-PTP gene, which encodes a segment of 12 hydrophilic amino acid residues (Mosinger et al. Proc Natl Acad Sci U S A. 1992 Jan 15;89(2):499-503; Lorenzen et al. J. Cell Biol 1995 Nov;131(3):631-43). This transcript is the major gene product found in most human and mouse tissues
5 (Kamatkar et al. J. Biol Chem 1996 Oct 25;271(43):26755-61). TC-PTPb mRNA is less abundant in human cells and is absent in the mouse. The predicted protein contains an extra hydrophobic segment of 36 amino acids (residues 382-418) at its carboxyl terminus (Mosinger et al. Proc Natl Acad Sci U S A. 1992 Jan 15;89(2):499-503; Lorenzen et al. J Cell Biol. 1995
10 Nov;131(3):631-43).

TC-PTP Knock Out Mice

[0007] TC-PTP^{-/-} mice appear physically normal until 10-14 days of age, at which time; they progressively develop tissue mononuclear cell infiltrates (You-Ten, 1997). Elevated levels of IFN- γ can be measured at 19 d
15 of age (Heinonen, 2004), and the animals die between 21 and 35 d of age. TC-PTP^{-/-} mice display defective hematopoiesis and immune function, characterized by anemia and splenomegaly secondary to sequestration of erythrocytes and accumulation of myeloid cells (You-Ten, 1997).

[0008] TC-PTP was also shown to interact with TRAF2 downstream of
20 the TNF proinflammatory cytokine. This interaction inactivated Src and suppressed MAPK signaling (van Vliet, 2005). TC-PTP has been identified as a critical regulator of colony-stimulating factor 1 (CSF-1) signaling and mononuclear phagocyte development. Upon CSF-1 stimulation, a deficiency in TC-PTP leads to enhanced tyrosine phosphorylation of the
25 Grb2/Gab2/Shp2 complex by the CSF-1 receptor, and increased activation of Erk (Simoncic, 2006). These results identified TC-PTP as a key modulator of inflammatory signals, as well as macrophage and lymphocyte functions.

The JAK/STAT signaling pathway is important for the development of hematopoietic and endothelial cell lineages

- 4 -

[0009] TC-PTP has been shown to control cytokine signaling events by its negative action on the Janus kinase (Jak) and signal transducer and activator of transcription (Stat) pathways (reviewed in (Bourdeau, 2005). The JAK/STAT pathway is widely used by the cytokine receptor superfamily to link
5 receptor stimulation to gene transcription (Aaronson, 2002). This pathway is crucial for hematopoietic and endothelial development, as well as cellular response to growth factors (Aaronson, 2002). Using an in vitro approach, TC-PTP substrate-trapping mutant D/A was shown to interact with Jak1 and Jak3 (Simoncic, 2002). Stat1, Stat3 and Stat5a/5b were also identified as
10 substrates for TC-PTP (ten Hoeve, 2002; Yamamoto, 2002; Aoki, 2002).

Stem Cells

[0010] Hematopoietic stem cells (HSC) and endothelial progenitor cells (EPC) represent 0.05% of total bone marrow cellularity and are, at the present time, indistinguishable from each other. The existence of a common
15 precursor, termed the hemangioblast, remains controversial. Long-term HSC (LT-HSC) give rise to short-term HSC (ST-HSC) that have limited self-renewal activity. ST-HSC become committed and can differentiate into common myeloid progenitors (CMP) or common lymphoid progenitors (CLP). CLP further differentiate into all lymphoid lineages including mature T, B and NK
20 cells. CMP can give rise to granulocyte-monocyte progenitors (GMP), yielding mature monocytic and granulocytic populations, or to megakaryocyte-erythrocyte progenitors (MEP), which produce platelets and erythrocytes. EPC can differentiate into circulating EPC (CEPC) and tissue EPC (TEPC), ultimately giving rise to mature endothelial cells (EC) (see Figure 1).
25 Alternatively, monocytes may also differentiate into TEPC. Cell surface markers that define each marrow stem cell subpopulation are indicated. Figure 1 adapted from references (Akashi, 2000; Hristov, 2004; Iwami, 2004; Kondo, 1997; Urbich, 2004; Urbich, 2004).

Vasculogenesis and EPC

30 **[0011]** Vasculogenesis refers to *de novo* formation of blood vessels from undifferentiated precursor cells. This process contributes to postnatal

- 5 -

neovascularization, and may be mediated by bone marrow-derived EPC or HSC (reviewed in ^{11,12}). The bone marrow is the major reservoir for these progenitors. Specifically, these cells can differentiate into endothelial cells, forming capillary-like structures *in vitro* and acquiring mature endothelial cell markers such as CD31 ¹³⁻¹⁵. A small percentage of EPC/HSC are released into the circulation (CEPC) and incorporate the vessel structure as a normal process of vascular regeneration after local injury. This vessel regeneration can be enhanced by augmenting the local availability of EPC. Indeed, the transplantation of *ex vivo* expanded EPC or HSC population leads to increased capillary density and better blood flow recovery after an ischemic event. This functional improvement has been investigated in a murine model of peripheral ischemia, namely hind limb ischemia with femoral artery ligation ¹⁶⁻¹⁸, and after myocardial infarction (MI) following coronary artery ligation ^{19,20}.

Regulating EPC number and function

[0012] The relative number of CEPC in peripheral blood is below 0.1% in normal conditions. However, following ischemic injury, EPC are mobilized from the bone marrow and are preferentially recruited to sites of ischemia, where they are incorporated into new blood vessels. This increased number of EPC found at the site of injury correlates with increased cytokine concentrations ²¹⁻²⁴ and the activation of STAT3 and STAT5 in vascular smooth muscle cells and in hypoxic endothelial cells, respectively ²⁵⁻²⁷. In humans, cytokines such as G-CSF increase the number of circulating CD34⁺ stem cells by enhancing mobilization and recruitment of EPC from the bone marrow ²⁸. In animal models, G-CSF improved cardiac function after MI ²⁹. Another cytokine, GM-CSF is also known to increase the number of circulating hematopoietic progenitors in humans, including EPC, as shown by improvement of hind limb neovascularization using the hind limb ischemia model ^{23,30}. Other factors such as vascular endothelial growth factor, angiopoietins, fibroblast growth factors, stromal-derived growth factors and HMG CoA reductase inhibitors also promote the mobilization and incorporation of EPC into new blood vessels ^{23,24,31-33}.

- 6 -

[0013] Agents for, and methods of augmenting local EPC numbers would be useful.

Summary of the invention

- 5 **[0014]** The inventors have demonstrated that abrogating T-cell protein tyrosine phosphatase (TC-PTP) activity increases stem cell populations including hematopoietic stem cell and endothelial progenitor cell populations. In addition the inventors have made the novel finding that the CD105 cell marker can be used to isolate and/or enrich for endothelial progenitor cells.
- 10 **[0015]** Accordingly the present invention provides a method for expanding stem cells comprising administering an effective amount of an agent that inhibits TC-PTP to a starting population of cells comprising stem cells whereby inhibition of TC-PTP increases stem cell expansion in a final population of cells.
- 15 **[0016]** Additionally the present invention provides a method for isolating expanded stem cells comprising:
- a) administering an effective amount of an agent that inhibits TC-PTP to a starting population of cells comprising stem cells whereby inhibition of TC-PTP increases stem cell expansion in a final population of cells; and
 - 20 b) isolating a population of expanded stem cells.
- [0017]** The present invention also provides a use of a substance that binds CD105 to isolate EPC. The invention further provides a method for isolating a population of endothelial progenitor cells comprising:
- 25 a) obtaining a starting population of cells comprising stem cells; and
 - b) isolating a final population of CD105+ cells wherein said final population of CD105+ cells comprise endothelial progenitor cells.

- 7 -

[0018] Further, the invention provides an isolated stem cell that has been expanded and/or isolated using a method of the invention.

[0019] Also provided is a culture medium for propagating stem cells including hematopoietic stem cells and endothelial progenitor cells, wherein
5 said culture medium comprises an effective amount of an agent that inhibits TC-PTP.

[0020] The present invention includes a method of identifying substances which can inhibit TC-PTP comprising the steps of:

- 10 (a) reacting TC-PTP and a test substance, under conditions that permit the test substance to interact with TC-PTP; and
- (b) assaying for TC-PTP activity or expression, wherein decreased TC-PTP activity or expression relative to control indicates the test substance is capable of
15 inhibiting TC-PTP.

[0021] In addition, the invention provides for the therapeutic use of stem cells, hematopoietic stem cells or endothelial progenitor cells that have been isolated or cultured using a method of the invention. Other embodiments provide therapeutic use of agents that inhibit TC-PTP for increasing stem cells
20 in an animal in need thereof. Methods of treatment using an agent that inhibits TC-PTP are also provided.

[0022] Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples
25 while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

- 8 -

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] The invention will now be described in relation to the drawings in which:

[0024] **Figure 1 schematically shows hematopoietic and endothelial differentiation in murine adult bone marrow.**

[0025] **Figure 2 shows increased numbers of EPC in TC-PTP^{-/-} bone marrow.** (A) TC-PTP^{+/+} and TC-PTP^{-/-} bone marrow cells were stained with antibodies to lineage (Lin) markers, and to CD105, CD117 and Sca-1, and analyzed by flow cytometry as described in *Materials and Methods*. The absolute cell count for the Lin⁻CD117⁺ population per 1 X 10⁶ bone marrow cells is indicated (*left panels*). The Lin⁻CD117⁺Sca-1⁺ population was further fractionated into 4 subsets (I-IV) based on the level of expression of Sca-1 (low or high), and on surface expression of CD105; the percentage of cells in each subset is indicated (*right panels*). (B) Absolute cell counts (per 1 X 10⁶ bone marrow cells) for the total Lin⁻CD117⁺ population and its CD105⁻ and CD105⁺ subsets were obtained by flow cytometry. P < 0.005. (C) Whole bone marrow was harvested from TC-PTP^{+/+} and TC-PTP^{-/-} mice, and whole bone marrow or fractionated stem cell subpopulations (CD105⁺ and CD105⁻) were incubated in EndoCult culture medium to allow the development of endothelial cell colonies (CFU-EC), as described in *Materials and Methods*. * p < 0.001. (D) Endothelial cell colonies (CFU-EC) obtained from TC-PTP^{+/+} and TC-PTP^{-/-} whole bone marrow were harvested at day 5 and analyzed by flow cytometry using endothelial cell surface markers.

[0026] **Figure 3 shows increased bone marrow CEPC and peripheral blood stem cells in TC-PTP^{-/-} bone marrow.** (A) Expression of CD14 was assessed in the Lin⁻CD117⁺Sca-1^{+/hi}CD105⁺ subpopulation by flow cytometry. RCN: relative cell number. P < 0.01. (B) TC-PTP^{+/+} and TC-PTP^{-/-} white blood cells were stained with antibodies to lineage (Lin) markers, and to CD117 and Sca-1, and analyzed by flow cytometry as described in *Materials and Methods*. EPC are identified as Lin⁻CD117⁺Sca-1^{+/hi} and the absolute cell counts per 1 X 10⁶ white blood cells is indicated (*square*). The Lin⁻

- 9 -

CD117⁺Sca-1^{+/hi} population was further fractionated based on the surface expression of CD105 and CD14, identifying CEPC. The absolute cell counts per 1×10^6 white blood cells is indicated (*square*). The representative data shown has been gated on Lin⁻ cells. (C) Peripheral blood was harvested from

5 TC-PTP^{+/+} (n = 6) and TC-PTP^{-/-} (n = 2) mice, and incubated in EndoCult culture medium to allow the development of endothelial cell colonies (CFU-EC), as described in *Materials and Methods*. * p < 0.001. Giemsa stained colonies are also shown in a representative experiment.

[0027] Figure 4. Increased phosphorylation of the CD117 receptor

10 **in TC-PTP^{-/-} bone marrow stem cell population.** (A, C) Whole bone marrow was harvested from TC-PTP^{+/+} and TC-PTP^{-/-} mice. *Ex vivo* cells were stained for surface expression of Lin⁻CD117⁺Sca-1^{+/hi} (identifying EPC; panel A) or Lin⁻CD117⁺Sca-1^{+/hi} CD105⁺ CD14⁺ (identifying CEPC; panel C), and intracellular expression of phosphorylated CD117 (p-CD117; phospho c-kit

15 receptor; thick line) or intracellular nonspecific IgG (thin line), for flow cytometry analysis. Contour plot analysis (left panels) were gated on Lin⁻ cells (EPC; panel A) and on Lin⁻CD117⁺Sca-1^{+/hi} (CEPC; panel C). Histograms (middle panels) are showing mean fluorescent intensity (MFI) of p-CD117 (thick line) compared to IgG (thin line) on EPC or CEPC. Histograms (right

20 panels) are showing MFI of total CD117 protein either in p-CD117 reaction (thick line) or in IgG reaction (thin line) on EPC or CEPC. (B, D) For EPC (panel B) and CEPC (panel D), the average MFI of IgG, p-CD117 and total CD117 protein (calculated in the IgG or p-CD117 reaction) are shown for TC-PTP^{+/+} (white bars; n = 6 for EPC; n = 4 for CEPC) and TC-PTP^{-/-} (black bars;

25 n = 5 for EPC; n = 4 for CEPC) mice. * p < 0.05.

[0028] Figure 5 shows increased stem cell population after treatment of whole bone marrow with sodium orthovanadate. Figure 6 shows treatment of murine bone marrow cells with TC-PTP blocking agents augments EPC population. (A, D) Balb/c, TC-PTP^{+/+} or PTP1B^{-/-}

30 bone marrow cells were electroporated with a control scramble sequence (SCR) or TC-PTP specific RNAi sequence (panel A) or cultured in the

- 10 -

presence of indicated concentrations of small molecule inhibitor (panel D). Both culture types were analyzed by flow cytometry 48 h post-treatment. Cells were stained with antibodies to lineage (Lin) markers, and to CD117 and Sca-1, as described in *Materials and Methods*. Contour plot analysis was gated on Lin⁻ cells and the absolute EPC count (per 1 X 10⁶ marrow cells) for the CD117⁺Sca-1⁺ fraction is indicated. (B, E) The average number of stem cells (per 1 X 10⁶ marrow cells) are shown for both the RNAi experiments (panel B) and the small molecule inhibitor (panel E). Results obtained with Balb/c bone marrow are represented by white bars (n = 3), from TC-PTP^{+/-} bone marrow with gray bars (n = 3) and from PTP1B^{-/-} bone marrow with black bars (n = 3). * p < 0.05. (C, F) Balb/c bone marrow was harvested, treated with either RNAi sequence (panel C) or with small molecule inhibitor (panel F) and incubated in EndoCult culture medium to allow the development of endothelial cell colonies (CFU-EC), as described in *Materials and Methods*. * p < 0.001.

[0029] Figure 7. Treatment of human bone marrow cells with TC-PTP blocking agents augments EPC population. (A, C) Human bone marrow cells were electroporated with a control scramble sequence (SCR) or TC-PTP specific RNAi sequence (panel A) or cultured in the presence of indicated concentrations of small molecule inhibitor (panel C). Both culture types were analyzed by flow cytometry 48 h post-treatment. Cells were stained with Lin⁻CD34⁺CD133⁺CD117⁺ to identify EPC and Lin⁻CD34⁺CD133⁺CD117⁺CD105⁺CD14⁺ for CEPC as described in *Materials and Methods*. Contour plot analysis was gated on Lin⁻CD34⁺ cells (EPC) or Lin⁻CD34⁺CD133⁺CD117⁺ (CEPC) and the absolute EPC or CEPC count (per 1 X 10⁶ marrow cells) is indicated. (B, D) The average number of stem cells (per 1 X 10⁶ marrow cells) are shown for both the RNAi experiments (panel B) and the small molecule inhibitor (panel D). Results obtained for EPC are represented by white bars (n = 4) and for CEPC with black bars (n = 2). * p < 0.05.

DETAILED DESCRIPTION OF THE INVENTION

- [0030]** The inventors have demonstrated that abrogating T cell protein tyrosine phosphatase (TC-PTP) activity increases stem cell populations including hematopoietic stem cell (HSC) and endothelial progenitor cell (EPC) populations, including circulating EPC (CEPC). The inventors have demonstrated, using a variety of agents that inhibit TC-PTP including gene ablation, RNAi silencing, non-selective phosphatase inhibitors (including both inorganic molecules such as sodium orthovanadate and conventional small organic molecules) that inhibit TC-PTP are useful for augmenting stem cell populations including HSC, EPC and CEPC populations. The inventors have demonstrated these results in a variety of cells including murine and human cells. The inventors have further demonstrated that EPC and CEPC are increased in the bone marrow and in the periphery where they can promote vasculogenesis.
- [0031]** In addition the inventors have made the novel finding that the CD105 cell surface marker can be used to isolate and/or enrich endothelial progenitor cells. The inventors have also shown that mouse stem cells expanded by a method of the invention, can be isolated using a method that comprises fractionation according to the level of Sca-1 expression and CD117 expression. Murine CEPC can be isolated using these and CD14. Further, the inventors show that expanded human stem cells, including EPC can be isolated using cell surface markers such as CD133, CD34, and CD117 and human CEPC can be isolated using these and CD14.
- [0032]** The results demonstrate a role for TC-PTP in regulation of both hematopoietic and endothelial stem cell proliferation, and suggest the therapeutic use of agents that inhibit TC-PTP to augment stem cell populations.
- [0033]** Accordingly, the present invention provides methods of expanding stem cell populations including EPCs and HSCs using an agent that inhibits TC-PTP. The invention also provides methods for isolating stem cell populations including EPCs. In other embodiments, the invention provides

- 12 -

methods for using stem cells and endothelial progenitor cells for various therapeutic applications including revascularization of ischemic tissues.

I. Expansion of Stem Cells

5 **[0034]** In one embodiment the present invention provides a method for expanding stem cells, said method comprising:

a) administering an effective amount of an agent that inhibits TC-PTP to a starting population of cells comprising stem cells, whereby inhibition of TC-PTP increases stem cell expansion in a final population of cells.

10

[0035] The term "stem cells" as used herein means immature cells that are capable of giving rise to different cell types and/or capable of differentiation and includes but is not limited to totipotent cells, hemagioblasts, pluripotent cells and multipotent progenitor cells, including endothelial progenitors cells, circulating endothelial progenitor cells, short-term hematopoietic cells and long-term hematopoietic cells.

15

[0036] "Endothelial progenitor cell" or EPC is used interchangeably with endothelial stem cell and is a progenitor cell or stem cell that gives rise to endothelial cells. EPC includes circulating EPC (CEPC) and tissue EPC (TEPC). EPC can be characterized by EPC cell marker expression. For example, a murine EPC is optionally characterized by Lin-CD117+Sca-1+/hiCD105+ expression and a human EPC is optionally characterized by Lin-CD34+CD133+CD117+CD105+. Further, CEPC are characterized by surface expression of CD14 in addition to EPC markers.

20

[0037] "Hematopoietic stem cell" or HSC is a stem cell that gives rise to hematopoietic cells and includes but is not limited to, long-term HSC (LT-HSC) and short-term HSC (ST-HSC).

25

[0038] The term "expanding stem cells" as used herein means that the number of stem cells is higher in the final cell population after administering

- 13 -

the agent that inhibits TC-PTP as compared to the starting population of cells. In one embodiment the total number of stem cells is increased 1.5 fold, preferably 2 fold, more preferably 2-3 fold, or 5 fold over the numbers in the starting cell population.

5 **[0039]** The term "a starting population of cells" as used herein can be comprised in an animal or any sample that contains stem cells including, but not limited to, blood, bone marrow, umbilical cord, lymphoid tissue, epithelia, thymus, liver, spleen, cancerous tissues, lymph node tissue, infected tissue, fetal tissue and fractions or enriched portions thereof. The starting population
10 or sample is preferably bone marrow or blood including peripheral blood or umbilical cord blood or fractions thereof, including buffy coat cells, mononuclear cells and low density mononuclear cells (LDMNC). For in vivo methods, the starting population is optionally patient blood or bone marrow, or any tissue sample that may be obtained for purposes of culture, or cell
15 fractionation for purification of resident stem cells followed by culture.

[0040] The term "animal", as used herein includes all members of the animal kingdom, especially mammals, including human. The animal is preferably human such as a patient.

[0041] The sample comprising the starting population can be obtained
20 using techniques known in the art.

[0042] The phrase "final population of cells" as used herein refers to the post treatment population of cells that comprises augmented or increased stem cells, such as EPC and HSC, as compared to the starting population of cells. "Post treatment" as used herein refers to post administration of an agent
25 that inhibits TC-PTP. The final population in certain embodiments is bone marrow. In other embodiments, the final population is peripheral blood. The final population of cells can also be an ex vivo treated tissue or cell culture.

[0043] For ex vivo expansions, prior to incubating the starting population of cells derived from the starting sample or fraction thereof, with
30 the agent that inhibits TC-PTP, the starting sample or fraction thereof may be

- 14 -

enriched for certain cell types and/or depleted for other cell types. In particular, the starting sample or fraction thereof may be enriched for endothelial progenitor cells and/or depleted of mature cells and/or other multipotent progenitor cells. The sample may be enriched or depleted of certain cell types using techniques known in the art. In one embodiment, the cells of a particular phenotype may be depleted by incubating the starting sample or fraction thereof with a substance that binds a cell marker or substances that bind cell markers such as an antibody cocktail containing antibodies specific for markers on the cells to be depleted.

10 **[0044]** The antibody cocktail can comprise one or more antibodies to specific cell markers present on cells to be depleted. Cell markers that may be used to deplete a starting sample or fraction thereof include but are not limited to "terminal differentiation" cell markers and multipotent progenitor cell markers. "Terminal differentiation" cell markers are expressed on mature or differentiated cell lineages and multipotent progenitor cell markers are expressed on, but are not limited to, ST-HSC, CLP, CMP, GMP, and MEP (see figure 1). Terminal differentiation cell markers suitable for use in the present invention comprise but are not limited to CD3, CD4, CD5, CD8, CD11b, CD19, CD31, CD49b, Ter119; and multipotent progenitor cell markers suitable for use in the present invention comprise but are not limited to CD127 and CD135. Other names for these markers are provided in Table 1. In addition, a person skilled in the art will recognize that other markers that are expressed on differentiated cell lineages and multipotent progenitors can also be used with the methods of the invention. Ter119 is a murine marker of erythroid cells. As indicated in Table 1, CD235a can be used to replace this marker in humans. The term "Lin markers" is used interchangeably with terminal differentiation cell markers. The Lin marker antibody cocktail is also enriched with antibodies to multipotent progenitor cell markers. A person of ordinary skill in the art would understand which cell markers can be used to deplete a starting sample. Further, such a person would recognize that cell marker homologues or orthologues or functionally equivalent cell markers may be used with cells of the corresponding species.

- 15 -

[0045] In another embodiment, the cells of a particular phenotype may be enriched by incubating a starting sample or fraction thereof with an antibody cocktail containing antibodies specific for markers on the cells to be enriched. The cells to be enriched are a population of stem cells. In a preferred embodiment, the cells to be enriched are EPC. In another embodiment, the cells to be enriched are HSC.

[0046] Cell markers that may be used to enrich a starting sample of the present invention include but are not limited to CD133, CD34, CD117, Sca-1 and CD105. Sca-1 is a murine stem cell marker. As indicated in Table 1, CD133 and CD34 can be used in humans. CD117 and Sca-1 can be used to enrich for murine HSC and EPC. CD117, CD133 and CD34 can be used to enrich for human HSC and EPC. Accordingly in certain embodiments, cells expressing CD117 and Sca-1 cell markers are enriched by incubating a starting sample or fraction thereof with an antibody cocktail containing antibodies specific for CD117 and Sca-1. In another embodiment, cells expressing CD117, CD133 and/or CD34 cell markers are enriched by incubating a starting sample or fraction thereof with an antibody cocktail containing antibodies specific for CD117, CD133 and/or CD34.

[0047] The cell marker CD105 can be used to further enrich for EPC. In a preferred embodiment, EPCs are enriched by incubating a starting sample or fraction thereof with an antibody cocktail containing antibodies specific for CD105. In another embodiment, in the mouse, the antibody cocktail comprises antibodies specific for CD105, CD117 and Sca-1.

[0048] Furthermore, other cell markers can also be used to enrich a starting population. Other cell markers that can be used comprise CD34 and CD133. Accordingly, in a further embodiment, in humans, the antibody cocktail comprises antibodies specific for CD105, CD117, CD133 and CD34. One skilled in the art would readily recognize which cell markers would be useful and which antibodies could be used or combined in an antibody cocktail to enrich a starting population of cells.

- 16 -

[0049] The cell marker CD14 can be used to further enrich for circulating EPC (CEPC). In one embodiment, CEPCs are enriched by incubating a starting sample or fraction thereof with an antibody cocktail containing antibodies specific for CD14. In another embodiment, in the
5 mouse, the antibody cocktail comprises antibodies specific for CD14, CD105, CD117 and Sca-1. In a further embodiment, in humans, the antibody cocktail comprises antibodies specific for CD14, CD105, CD117, CD133 and CD34.

[0050] Antibodies specific for a particular cell marker are preferably used to deplete and/or enrich a starting sample of particular cell types. Other
10 molecules that bind cell markers may optionally be used, such as aptamers. Molecules that bind cell markers are typically tagged with a detection unit. The detection unit can comprise a fluorochrome tag and permits cells that have bound the molecule to be separated or fractionated by flow cytometry. Flow cytometry techniques are well known to persons skilled in the art.

[0051] One skilled in the art would recognize that other methods known in the art could also be used to deplete and/or enrich for specific cell types. For example, magnetic beads can be used to enrich or deplete a starting population of cells. Methods employing magnetic beads include but are not limited to use of commercially available products such as EasySep or
20 CellSep.

[0052] Another method employs ficoll or other similar separation gradients to isolate bone marrow mononuclear cells. Further, it will be readily recognized by one skilled in the art that such methods can be further combined resulting in progressive sounds of enrichment. In one embodiment
25 a starting population is enriched using magnetic beads. In an alternate embodiment a starting population is depleted using magnetic beads. In other embodiments, methods employing magnetic beads are combined with other enrichment and/or depletion methods. In a further embodiment, the other enrichment and/or depletion method comprises flow cytometry methods. In
30 another aspect, depletion and/or enrichment of a starting population comprises separation of cells using ficoll gradients. In other embodiments,

- 17 -

methods employing ficoll are combined with other enrichment and/or depletion methods. In one embodiment flow cytometry is the other enrichment or depletion method.

[0053] Additionally, increased stem cell expansion can be detected
5 using stem cell markers such as HSC and/or EPC stem cell markers and methods known in the art such as flow cytometry.

The term "TC-PTP" refers to T-cell protein tyrosine phosphatase and includes but is not limited to variants and homologs of TC-PTP from all species and sources. For example as used herein TC-PTP includes but is not limited to
10 TC-PTPa and TC-PTPb. The term TC-PTP includes but is not limited to a TC-PTP gene (*ptpn2*), protein, mRNA transcript or cDNA. Example of TC-PTP include but are not limited to mouse TC-PTP sequences NW_000134, NW 001030635, NT 039674 and human TC-PTP sequences NT 010859, NW 926940.

[0054] The term "an agent that inhibits TC-PTP" is used herein interchangeably with "TC-PTP inhibitor" and/or "TC-PTP blocking agent" and means an agent that inhibits TC-PTP including all variants and homologs of TC-PTP from all species and sources. Agents that inhibit TC-PTP comprise agents that inhibit TC-PTP enzyme activity, as reflected for example in
20 increased phosphorylation of downstream molecules such as CD117, agents that reduce TC-PTP protein expression, agents that reduce TC-PTP mRNA expression and agents that prevent normal intracellular localization or promote the intracellular mislocalization of TC-PTP affecting its normal function. Agents that inhibit TC-PTP comprise pharmacological and biological
25 agents including antisense nucleic acids and RNAi nucleic acid molecules. "An agent that inhibits TC-PTP" may comprise one or more TC-PTP inhibitors.

A) TC-PTP Inhibitors

(i) Phosphatase and Small Molecule Inhibitors

- 18 -

[0055] One class of TC-PTP inhibitor comprises phosphatase inhibitors. As shown by the inventors, the phosphatase inhibitor can be a non-specific phosphatase inhibitor. The phosphatase inhibitor is optionally an organic molecule or inorganic molecule. In one embodiment, the agent that inhibits TC-PTP comprises a tyrosine phosphatase inhibitor. In a more specific embodiment, the tyrosine phosphatase inhibitor is a vanadium containing tyrosine phosphatase inhibitor. Vanadium containing tyrosine phosphatase inhibitors include but are not limited to vanadate, orthovanadate, pervanadate; vanadate dimer, vanadate tetramer, vanadate pentamer, vanadate hexamer, vanadate heptamer, vanadate octamer, vanadate nonamer, vanadate decamer, vanadate polymer, vanadyl sulfate, bis (6, ethylpicolinate) (H₂O) oxovanadium(IV) complex, bis (1-oxy-2-pyridinethiolato) oxovanadium (IV), bis (maltolato) oxovanadium (IV), bis (biguanidato) oxovanadium (IV), bis (N'N'-dimethylbiguanidato) oxovanadium (IV), bis (beta-phenethyl-biguanidato) oxovanadium (IV), and any salts thereof.

[0056] In another more specific embodiment the vanadium tyrosine phosphatase inhibitor is orthovanadate or a salt thereof. The concentration of orthovanadate is preferably 10-100 μ M. More preferably the concentration is 10-50 μ M.

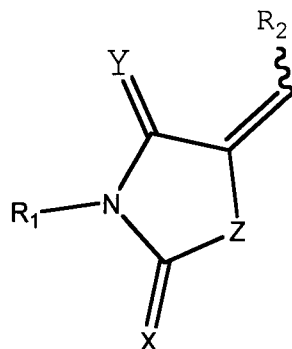
ii) Small Molecule Inhibitors

[0057] An agent that inhibits TC-PTP is in one embodiment a small molecule inhibitor.

Thioxothiazolidinone Compounds

[0058] One class of small molecule inhibitor that inhibits TC-PTP comprises thioxothiazolidinone compounds. The inventors have previously identified thioxothiazolidinone compounds that inhibit PTP1B⁴⁰. The inventors now show that these compounds inhibit TC-PTP activity and are useful for augmenting stem cell populations. Thioxothiazolidinone compounds useful for augmenting stem cells comprise compounds of Formula I:

- 19 -



Formula I

or a pharmaceutically acceptable salt thereof, or prodrugs thereof, wherein:

X and Y are independently an oxygen or sulfur;

5 Z is sulfur, oxygen, nitrogen or a methylene group;

R₁ is H, an alkyl, a cycloalkyl, a substituted or unsubstituted cycloalkenyl, an aryl, a m-halophenyl, a p-halophenyl, a m-alkylphenyl, a m-alkoxyphenyl, a p-alkylphenyl, a p-alkoxyphenyl, a hydroxyphenyl, a dichlorophenyl, a pyrrole, a furan, a pyridine, a piperazine, or a morpholino
 10 ring group; and R₂ is a substituted aryl, a substituted or unsubstituted cycloalkyl, or a substituted or unsubstituted cycloalkenyl.

[0059] Accordingly, in one embodiment the agent that inhibits TC-PTP is a thioxothiazolidinone compound. In one embodiment, the alkyl of R₁ is a C₁₋₁₀ alkyl. In another embodiment, the cycloalkyl of R₁ is a C₃₋₈ cycloalkyl. In
 15 a further embodiment, the cycloalkenyl of R₁ is a C₄₋₈. In a still further embodiment, the aryl of R₂ has 3-nitro, 4-hydroxy, and 5-methoxybenzene substituents; 3-nitro, 4,5-dihydroxy benzene substituents; 3-nitro, 4-hydroxy, 5-alkoxy substituents; or 3-nitro, 4-hydroxy, 5-cycloalkyl substituents. In particular embodiments, the 5-alkoxy is a C₂₋₈ alkoxy. In other embodiments,
 20 the 5-cycloalkyl is a C₃₋₈ cycloalkyl.

[0060] The chiral centers of carbon atoms of compounds of Formula I can independently of one another have R or S configurations.

[0061] Further, it is contemplated that enantiomers, isomers or tautomers, as well as any derivatives or analogs of compounds of Formula I
 25 that retain the same biological activity of inhibiting TC-PTP will be useful as

- 20 -

agents for methods disclosed herein. For example, a halogen group of a compound provided herein can be substituted with another halogen group such as a fluoro, chloro, bromo or iodo group and retain biological activity.

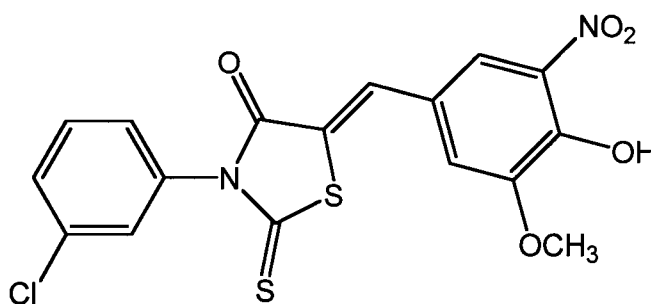
- [0062]** A pharmaceutically acceptable salt of a compound of Formula I
- 5 can be obtained using methods well-known to those skilled in the art. For example, a salt can be obtained by combining a compound of Formula I with an inorganic or organic acid or base in a solvent or diluent, or from other salts by cation exchange or anion exchange. Salt-forming groups in a compound of Formula I are groups or radicals having basic or acidic properties.
- 10 Compounds having at least one basic group or at least one basic radical, can form acid addition salts with, for example, inorganic acids such as hydrochloric acid, sulfuric acid, a phosphoric acid, or with suitable organic carboxylic or sulfonic acids. Suitable organic carboxylic or sulfonic acids may include aliphatic mono- or di-carboxylic acids (e.g., trifluoroacetic acid, acetic acid, propionic acid, glycolic acid, succinic acid, maleic acid, fumaric acid,
- 15 hydroxymaleic acid, malic acid, tartaric acid, citric acid, oxalic acid); amino acids (e.g., arginine, lysine); aromatic carboxylic acids (e.g., benzoic acid, 2-phenoxy-benzoic acid, 2-acetoxy-benzoic acid, salicylic acid, 4-aminosalicylic acid); aromatic aliphatic carboxylic acids (e.g., mandelic acid, cinnamic acid);
- 20 heteroaromatic carboxylic acids (e.g., nicotinic acid, isonicotinic acid); aliphatic sulfonic acids (e.g., methane-, ethane- or 2-hydroxyethane-sulfonic acid) or aromatic sulfonic acids (e.g., benzene-, *p*-toluene- or naphthalene-2-sulfonic acid). When several basic groups are present, mono- or poly-acid addition salts may be formed. Compounds of the invention having acidic
- 25 groups, e.g., a free carboxy group, can form metal or ammonium salts such as alkali metal or alkaline earth metal salts (e.g., sodium, potassium, magnesium or calcium salts) or ammonium salts with ammonia or suitable organic amines such as tertiary monoamines (e.g., triethylamine or tri-(2-hydroxyethyl)-amine), or heterocyclic bases (e.g., N-ethyl-piperidine or N,N'-
- 30 dimethylpiperazine).

- 21 -

[0063] By pharmaceutically acceptable is meant a material that is not biologically or otherwise undesirable, *i.e.*, the material can be administered to a subject without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

[0064] Use of prodrugs of compounds of Formula I are also embraced by the present invention. A prodrug of Formula I or a pharmaceutically acceptable salt thereof is intended to include any covalently bonded carrier which releases the active parent drug according to Formula I *in vivo* when such prodrug is administered to a mammalian subject. Prodrugs of a compound of Formula I are prepared by modifying functional groups present in the compound in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent compound. Prodrugs include compounds of Formula I wherein a hydroxy, amino, or sulfhydryl group is bonded to any group that, when the prodrug of a compound of Formula I is administered to a mammalian subject, cleaves to form, for example a free hydroxyl.

[0065] By way of illustration, incubation of a starting population of cells with an uncharged bis-aryl thioxothiazolidinone compound, specifically 5-[(4-hydroxy-3-methoxy-5-nitrophenyl)methylene]-3-(*m*-chlorophenyl)-2-thioxo-4-thiazolidinone (compound 1) was found to increase stem cell numbers. Compound 1 has the following formula:



Compound 1

- 22 -

[0066] Accordingly, in one embodiment the agent that inhibits TC-PTP is compound 1. Compound 1 inhibits TC-PTP with an IC₅₀ of 5.3±0.1 at pH 7.0⁴⁰.

[0067] For therapeutic use, compounds of Formula I or
5 pharmaceutically acceptable salts thereof, or prodrugs thereof are generally combined with pharmaceutically acceptable carriers before use. Examples of such carriers and methods of formulation of pharmaceutically acceptable compositions containing inhibitors and carriers can be found in Remington: The Science and Practice of Pharmacy, Alfonso R. Gennaro, editor, 20th ed.
10 Lippincott Williams & Wilkins: Philadelphia, PA, 2000. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the inhibitor.

[0068] In view of the structural homology among protein tyrosine phosphatases and especially between PTP-1b and TC-PTP (Bourdeau A. et al. Curr Opin Cell Biol. 2005 Apr;17(2):203-9), an inhibitor of PTP-1b may
15 also inhibit TC-PTP. Thus a non-selective inhibitor of PTP-1b may also be useful for inhibiting TC-PTP.

(iii) Nucleic Acids

[0069] Agents that inhibit TC-PTP also comprise nucleic acid molecules
20 that reduce TC-PTP mRNA levels, TC-PTP protein expression and/or that directly inhibit TC-PTP activity.

[0070] The term "nucleic acid molecule" as used herein refers to an oligomer or polymer of nucleotide or nucleoside monomers consisting of naturally occurring bases, sugars, and intersugar (backbone) linkages. The
25 term also includes modified or substituted oligomers comprising non-naturally occurring monomers or portions thereof, which function similarly. Such modified or substituted nucleic acid molecules may be preferred over naturally occurring forms because of properties such as enhanced cellular uptake, or increased stability in the presence of nucleases. The term also includes
30 chimeric nucleic acid molecules that contain two or more chemically distinct

- 23 -

regions. For example, chimeric nucleic acid molecules may contain at least one region of modified nucleotides that confer beneficial properties (e.g. increased nuclease resistance, increased uptake into cells), or two or more nucleic acid molecules of the invention may be joined to form a chimeric
5 nucleic acid molecule.

[0071] The nucleic acid molecules of the present invention may be ribonucleic or deoxyribonucleic acids and may contain naturally occurring bases including adenine, guanine, cytosine, thymidine and uracil. The nucleic acid molecules may also contain modified bases such as xanthine,
10 hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl adenines, 5-halo uracil, 5-halo cytosine, 6-aza uracil, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiouracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8-amino guanine, 8-thiol guanine, 8-thiolalkyl guanines, 8-
15 hydroxyl guanine and other 8-substituted guanines, other aza and deaza uracils, thymidines, cytosines, adenines, or guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

[0072] Other nucleic acid molecules of the invention may contain modified phosphorous, oxygen heteroatoms in the phosphate backbone, short
20 chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages.

[0073] The nucleic acid molecules of the invention may also comprise nucleotide analogs that may be better suited as therapeutic or experimental reagents. An example of a nucleic acid molecule analogue is a peptide
25 nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in the DNA (or RNA), is replaced with a polyamide backbone which is similar to that found in peptides (P.E. Nielsen, et al Science 1991, 254, 1497). PNA analogues have been shown to be resistant to degradation by enzymes and to have extended lives *in vivo* and *in vitro*. PNAs also bind stronger to a
30 complimentary DNA sequence due to the lack of charge repulsion between the PNA strand and the DNA strand. Other oligonucleotides may contain

- 24 -

nucleotides containing polymer backbones, cyclic backbones, or acyclic backbones. For example, the nucleotides may have morpholino backbone structures (U.S. Patent No. 5,034,506). Nucleic acid molecules may also contain groups such as reporter groups, a group for improving the pharmacokinetic properties of a nucleic acid molecule, or a group for improving the pharmacodynamic properties of a nucleic acid molecule. Nucleic acid molecules may also have sugar mimetics.

[0074] The nucleic acid molecules may be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art.

10 The nucleic acid molecules of the invention or a fragment thereof, may be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed with mRNA or the native gene (e.g. phosphorothioate derivatives and acridine substituted nucleotides). The nucleic acid molecules may be produced biologically using an expression vector introduced into cells in the form of a recombinant plasmid, phagemid or attenuated virus in which the nucleic acid molecules are produced under the control of a high efficiency regulatory region, the activity of which may be determined by the cell type into which the

15

20 vector is introduced.

[0075] The nucleic acid molecules of the invention include but are not limited to antisense nucleic acid molecules and double stranded nucleic acid molecules as well as nucleic acid molecules that inhibit the enzymatic activity of TC-PTP and which are unrelated to the sequence of TC-PTP.

25 **[0076]** The term "antisense nucleic acid molecule" as used herein means an oligomer or polymer of nucleotide or nucleoside monomers that is complementary to its target, e.g. the TC-PTP mRNA transcript. In one embodiment an agent that inhibits TC-PTP comprises an antisense nucleic acid molecule. The antisense nucleic acid molecule is minimally 15 nucleotides and can be 15-20, 20-30, 30-40, 40-50 or 50-100 nucleotides in length. The antisense nucleic acid molecule can be greater than 100

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- 25 -

nucleotides and is maximally the number of nucleotides present in a TC-PTP transcript.

[0077] The term "double stranded nucleic acid molecule" as used herein means a nucleic acid molecule comprising two strands of oligomers or polymers of nucleotide monomers, wherein the oligomers or polymers are electrostatically bonded. In one embodiment, the double stranded nucleic acid molecule comprises a nucleic acid molecule suitable for reducing TC-PTP expression by RNA interference (RNAi). The nucleic acid molecule suitable for RNAi methods may be double stranded RNA and may be an oligomer that is composed of 20-30 nucleotides, or greater than 30 nucleotides. Methods for RNAi technology are well known in the art.

[0078] The inventors have demonstrated that nucleic acid molecules for RNAi (e.g RNAi sequences) inhibition of TC-PTP increase stem cell numbers. For example incubating a starting population with TC-PTP directed RNAi sequence, having sense sequence GCCCAUAUGAUCACAGUCG (SEQ ID NO: 1) which targets exon 2 in human and mouse TC-PTP, increases EPC and CEPC numbers in human and mouse final populations. Similarly, incubating a starting population with TC-PTP directed RNAi sequence, having sense sequence GGCACAAAGAAGUUACAUC (SEQ ID NO: 2) which targets exon 3 in mouse TC-PTP, increases HSC, EPC and CEPC in mouse final populations. Scrambled RNAi sequences (SCR) did not increase EPC cell numbers. A person skilled in the art will realize that shorter, longer or modified RNAi sequences can be used. Shorter RNAi sequences, such as RNAi sequences at least 15 nucleotides are optionally used to inhibit TC-PTP. Two thymidine nucleotides are optionally added to the sequence to create a two nucleotide overhang. For example, the inventors have used TC1 having sequence GCCCAUAUGAUCACAGUCGtt (SEQ ID NO: 3) in mouse and human cells and TC2, having sequence GGCACAAAGAAGUUACAUCtt (SEQ ID NO: 4) in mouse cells to inhibit TC-PTP. It is known in the art that a efficient silencing is obtained with siRNA duplexes complexes paired to have a two nucleotide 3' overhang. Adding two thymidine nucleotides is thought to

- 26 -

add nuclease resistance. A person skilled in the art will recognize that other nucleotides can also be added.

- [0079]** Accordingly, in one embodiment the agent that inhibits TC-PTP is an RNAi sequence. In another embodiment the RNAi sequence is
5 GCCCAUAUGAUCACAGUCG (SEQ ID NO:1). In a further embodiment, the RNAi sequence is GGCACAAAGAAGUUACAUC (SEQ ID NO:2). In another embodiment the RNAi sequence is GCCCAUAUGAUCACAGUCGtt (SEQ ID NO:3). In a further embodiment, the RNAi sequence is GGCACAAAGAAGUUACAUCtt (SEQ ID NO:4). A person skilled in the art will
10 recognize that other RNAi sequences that target TC-PTP can be identified using methods known in the art and used in the methods described herein. Furthermore, as the inventors have shown, multiple RNAi sequences can be used simultaneously. RNAi sequences can be used in series to reduce TC-PTP levels in a starting population.
- 15 **[0080]** Nucleic acid molecules that are unrelated to the sequence of TC-PTP comprise aptamers. Aptamers are short strands of nucleic acids that can adopt highly specific 3-dimensional conformations. Aptamers can exhibit high binding affinity and specificity to a target molecule. These properties allow such molecules to specifically inhibit the functional activity of enzymes
20 and are included as agents that inhibit TC-PTP.

(iv) Antibodies

- [0081]** In another embodiment, the agent that can inhibit TC-PTP is a TC-PTP specific antibody. Antibodies to TC-PTP may be prepared using techniques known in the art such as those described by Kohler and Milstein,
25 Nature 256, 495 (1975) and in U.S. Patent Nos. RE 32,011; 4,902,614; 4,543,439; and 4,411,993, which are incorporated herein by reference. (See also Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Plenum Press, Kennett, McKearn, and Bechtol (eds.), 1980, and Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor
30 Laboratory Press, 1988, which are also incorporated herein by reference). Within the context of the present invention, antibodies are understood to

- 27 -

include but are not limited to monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, and F(ab')₂) and recombinantly produced binding partners.

(v) Other substances

5 **[0082]** In addition to phosphatase inhibitors, small molecule inhibitors, nucleic acids and antibodies, other substances that can inhibit TC-PTP can be identified and used in the methods of the invention. For example, substances which inhibit TC-PTP may be identified by reacting TC-PTP with a substance which potentially inhibits TC-PTP, then detecting if TC-PTP activity or
10 expression is reduced.

[0083] Accordingly, the present invention also includes a method of identifying substances which can inhibit TC-PTP comprising the steps of:

- 15 (c) reacting TC-PTP and a test substance, under conditions that permit the test substance to interact with TC-PTP;
 and
 (d) assaying for TC-PTP activity or expression, wherein decreased TC-PTP activity or expression relative to control indicates the test substance is capable of inhibiting TC-PTP.

20 **[0084]** Conditions which permit the substance and TC-PTP to interact may be selected having regard to factors such as the nature and amounts of the substance and the protein. The appropriate conditions are known to one skilled in the art.

25 **[0085]** An "effective amount of an agent that inhibits TC-PTP" means as used herein, an amount effective to inhibit TC-PTP activity or expression and can be determined using routine methods known to one of ordinary skill in the art. Further, two or more agents that inhibit TC-PTP may be used in combination. In addition, subsequent additions of an agent that inhibits TC-PTP may be added at various time points during the incubation of the starting
30 population of cells. In one embodiment an agent that inhibits TC-PTP is added one or more times after the initial incubation with an agent that inhibits TC-

- 28 -

PTP. Where the agent is administered in vivo, the agent may be administered one or more times after the initial administration. In addition the agent may be administered contemporaneously with one or more other agents that increase stem cell numbers and/or a therapeutic agent.

- 5 **[0086]** The term "administering" or "administration" as used herein, means that the starting population is exposed to an agent that inhibits TC-PTP under suitable conditions to expand the stem cells. The "administering" can be done in vitro or in vivo. The term "administering" as used herein, is synonymous with "incubating" especially for in vitro expansion of stem cells.
- 10 The "administering" can further comprise local or systemic administration of an agent that inhibits TC-PTP. Local administration is, in one embodiment, accomplished by direct injection. For example, in one embodiment, vanadate, a small molecule inhibitor such as compound 1, or a RNAi sequence specific for TC-PTP, is injected directly to a desired site. The injection is optionally an
- 15 intramuscular injection. The desired site is optionally an ischemic site such as a myocardial infarct site. Other methods of local administration are known in the art and discussed elsewhere herein. Systemic administration can be intravenous injection and/or other methods known in the art and discussed elsewhere herein.
- 20 **[0087]** In vitro, "suitable conditions" to expand the stem cells comprise using a suitable culture medium, in a suitable cell chamber, under suitable ambient conditions, for a suitable period of time to expand the stem cells.
- [0088]** A "suitable culture medium" is preferably any culture medium that supports stem cell growth. A suitable culture medium may comprise
- 25 animal serum, including fetal bovine serum (FBS), β -mercaptoethanol and/or antibiotics. In one embodiment, the culture medium is Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% FBS and 0.02% v/v β -mercaptoethanol. In another embodiment the culture medium is a long-term bone marrow culture medium. In one embodiment the long term bone marrow
- 30 culture medium is DMEM high glucose, 1% antibiotics 15% horse serum, 5% fetal calf serum, 10E-6 M hydrocortisone, 10E-4M β -mercaptoethanol,

- 29 -

transferrin 400µg/ml and WEHI supernatant (17%, final concentration). In another embodiment, EndoCult™ (Stem Cell Technologies) growth medium is also used. One skilled in the art would be aware of the different culture media available and their suitability for different stem cell cultures. For example a
5 skilled person would know that some animal sera may not be useful for propagating human stem cells which are to be introduced into a human. An alternative culture medium would be required.

[0089] "A suitable cell chamber" comprises a culture plate or flask that is permissive for maintaining stem cells. Different culture plates or flasks may
10 be used to incubate different starting samples or populations of cells and during different segments of the incubation. The types of cell chambers suitable for different starting samples or populations of cells are well known to someone skilled in the art. Similarly someone skilled in the art would know what cell chambers are suitable for different segments of the incubation. The
15 culture plate or flask may be coated to promote cell adherence of particular cell types. In one embodiment the suitable cell chamber is a plate or flask that is coated with fibronectin.

[0090] "Suitable ambient conditions" for expanding stem cells comprise maintaining cells with sufficient carbon dioxide, and at a suitable temperature.
20 Suitable ambient conditions may be maintained using an incubation unit that regulates carbon dioxide level and temperature. The suitable ambient conditions for expanding stem cells are known to a person skilled in maintaining in vitro cell cultures.

[0091] "A suitable period of time" is any period of time where inhibition
25 of TC-PTP increases the number of stem cells in the final cell population. A suitable period of time is minimally the minimum period of TC-PTP inhibition required to increase stem cell numbers. The suitable period of time will vary with the inhibitor. In one embodiment the suitable period is one cell cycle of a stem cell in the starting cell population. In one embodiment, the minimum
30 period of time is at least 12 hours or 24 hours. In a preferred embodiment, stem cells are expanded in the presence of a TC-PTP inhibitor for at least 24

- 30 -

hours. In another embodiment, stem cells are expanded in the presence of orthovanadate for at least 24 hours. In another embodiment, stem cells are incubated with or expanded in the presence of a small molecule inhibitor for at least 24 hours, 24-48 hours, or at least 48 hours. In one embodiment, the
5 small molecule inhibitor is a thioxothiazolidinone compound. In another embodiment, stem cells are incubated with or expanded in the presence of an RNAi sequence directed at TC-PTP. In one embodiment the RNAi sequence is TC1 (sense: GCCCAUAUGAUCACAGUCG (SEQ ID NO: 1), exon 2, human and mouse). In another embodiment, the RNAi sequence is TC2
10 (sense: GGCACAAAGAAGUUACAUC (SEQ ID NO: 2), exon 3, mouse). In a further embodiment, stem cells are incubated with or expanded in the presence of TC1 and TC2. Subsequently, stem cells may be cultured in the absence of a TC-PTP inhibitor.

II. Isolating Expanded Stem Cells

15 **[0092]** Additionally the present invention provides a method for isolating expanded stem cells comprising:

a) administering an effective amount of an agent that inhibits TC-PTP to a starting population of cells comprising stem cells, whereby inhibition of TC-PTP increases stem cell
20 expansion in a final population of cells; and

b) isolating a population of expanded stem cells from the final population of cells.

[0093] "Isolating" is used interchangeably with "fractionating" and as used herein refers to the separation of stem cells from the final population of
25 cells. A population of expanded stem cells may be isolated using cell markers.

[0094] Cell markers may be used to isolate or fractionate cells, from the final population of cells. Stem cells that are positive or express a particular cell marker or set of cell markers can be fractionated from the final population of cells using flow cytometry techniques, which are well established in the art.

- 31 -

[0095] Several cell markers are known to be associated with stem cells. CD117 and Sca-1 are cell markers that have previously been shown to be present on hematopoietic stem cells. Accordingly, cell markers that may be used to isolate a population of expanded stem cells of the present invention
5 comprise CD117 and Sca-1. In one embodiment CD117 and Sca-1 cell are markers are used to isolate expanded hematopoietic stem cells. In another embodiment, CD117 and Sca-1 are used to isolate expanded endothelial progenitor cells.

[0096] The inventors have made the novel finding that the cell marker
10 CD105 cells can be used to isolate and/or enrich for endothelial progenitor cells.

[0097] Accordingly in certain embodiments, CD105⁺ cells are used to isolate endothelial progenitor cells. In one embodiment the invention provides a method for isolating a population of expanded stem cells enriched for
15 endothelial precursor cells, said method comprising fractionating a final population of cells with cell markers comprising CD105. In another embodiment, the method comprises fractionating a final population of cells with stem cell markers comprising CD117, Sca-1 and CD105. Furthermore, other stem cell markers can also be used. Other stem cell markers include
20 but are not limited to CD34 and CD133. One skilled in the art would readily recognize which cell markers would be useful and which antibodies could be used or combined in an antibody cocktail to fractionate a final population of cells. It will be appreciated by one skilled in the art that the antibody cocktail can comprise various combinations of antibodies. Other antibodies to cell
25 markers not herein mentioned may be used and are considered within the scope of the invention.

[0098] In addition, cell markers can be used to separate stem cell populations that express different levels of a cell marker. A stem cell population may express a high level or a low level of a particular cell marker.
30 Flow cytometry techniques, which are well known in the art, can be used to

- 32 -

separate cells expressing a high level of a cell marker from cells expressing a low level of a cell marker.

[0099] The inventors have further shown that the level of Sca-1 expression can be used to isolate expanded stem cells. Accordingly in one embodiment, expanded stem cells are isolated using an antibody cocktail containing antibodies specific for the expression of Sca-1 and separating cells expressing a high level of Sca-1.

[00100] In addition cell markers can be used to deplete the final population of cells of certain cell types. Terminal differentiation cell markers and multipotent progenitor cell markers, previously described, can be used to isolate or fractionate stem cells that do not express or express low amounts of particular terminal differentiation cell markers and/or multipotent progenitor cell markers. Cell markers that are expressed on mature cell lineages and multipotent progenitors can also be used with the methods of the invention. A person of ordinary skill in the art would understand which cell markers can be used to deplete the final population of cells.

[00101] Isolating a population of expanded stem cells can comprise both a depletion step using terminal differentiation cell markers and/or multipotent progenitor cell markers and a positive selection step using cell markers expressed on stem cells.

[00102] Accordingly, in one embodiment the step of isolating a population of expanded stem cells comprises:

- i. fractionating a final population of cells to obtain a sub-population of cells negative for one or more terminal differentiation cell markers and/or multipotent progenitor cell markers; and
- ii. fractionating said sub-population of cells to obtain a stem cell enriched fraction using cell markers selected from the group CD117, Sca-1 and CD105 (in the mouse); or CD117, CD34, CD133 and CD105 (in the human).

- 33 -

[00103] In one embodiment, the final population is fractionated using terminal differentiation markers and multipotent progenitor cell markers, CD3, CD4, CD5, CD8, CD11b, CD19, CD49b, Ter119 or CD235a, CD31, CD127 and CD135 and the sub-population is fractionated using CD117 and Sca-1, or
5 CD117, CD133 and CD34, obtaining a stem cell enriched fraction comprising HSC. In a preferred embodiment, the final population is fractionated using terminal differentiation and multipotent progenitor markers, CD3, CD4, CD5, CD8, CD11b, CD19, CD49b, Ter119 or CD235a, CD31, CD127 and CD135 and the sub-population is fractionated using CD117, Sca-1 and CD105, or
10 CD117, CD133, CD34 and CD105, obtaining a stem cell enriched fraction comprising EPC.

[00104] The presence and relative expansion of EPCs obtained using a method of the invention can be determined by performing various endothelial progenitor cell colony-forming assays. The presence and relative expansion of
15 hematopoietic stem cells expanded using a method of the invention can be determined by performing various differentiation assays. These methods are routine and well known in the art to a person of ordinary skill.

III. Isolating Endothelial Progenitor Cells

[00105] The present invention also provides a method for isolating
20 endothelial progenitor cells and circulating endothelial progenitor cells, said method comprising:

- a) obtaining a starting population of cells comprising stem cells;
and
- b) isolating a final population of CD105+ cells wherein said final
25 population of CD105+ cells comprise endothelial progenitor cells.

[00106] As mentioned previously, CD105 is a cell marker that the inventors have shown can be used to isolate and/or enrich for endothelial progenitor cells. Other cell markers, including CD117 and Sca-1, CD133 and
30 CD34, can also be used in combination with CD105 to further isolate a

- 34 -

CD105+ enriched final population comprising endothelial precursor cells. Accordingly in one embodiment, the method comprises isolating CD105+, CD117+ and Sca-1+ cells. In a further more specific embodiment, Sca-1 expression is high.

- 5 **[00107]** Additionally terminal differentiation markers and multipotent progenitor cell markers can be used to deplete the starting population of particular cell types. In certain embodiments, the method comprises fractionating the starting population of cells comprising stem cells with terminal differentiation cell markers and/or multipotent progenitor cell markers.
- 10 In another embodiment, the method further comprises fractionating cells expressing CD117 and Sca-1. In a more specific embodiment, the method comprises fractionating cells expressing a high level of Sca-1.

IV. Stem Cells and Stem Cell Lines

- [00108]** The invention also provides for isolated stem cells that have
- 15 been expanded and/or isolated using a method of the invention. In one embodiment the isolated stem cell is a CD105+, CD117+ Sca-1+ EPC. In another embodiment, the isolated stem cell is a CD105+, CD117+, Sca-1+, CD14+ CEPC. In another embodiment, the isolated stem cell is a CD105+, CD117+, CD133+, CD34+ EPC. In another embodiment the isolated stem cell
- 20 is a CD105+, CD117+, CD133+, CD34+, CD14+ CEPC.

[00109] The invention provides in another embodiment, a method of establishing a stem cell line. In one embodiment, the stem cell line is a hematopoietic stem cell line. In another embodiment the stem cell line is an endothelial stem cell line.

- 25 **[00110]** One aspect of the invention provides a culture medium for propagating hematopoietic stem cells and/or endothelial progenitor cells, wherein said culture medium contains an agent that inhibits TC-PTP. In one embodiment the culture medium is a conditioned medium which is made from cells secreting virus comprising nucleic acid molecules that inhibit TC-PTP. In
- 30 another embodiment, the nucleic acid molecule comprises an antisense

- 35 -

molecule. In another embodiment the nucleic acid molecule comprises a molecule that inhibits TC-PTP through RNA interference. In another embodiment the culture medium and inhibiting agent are provided separately, to be combined either prior to use, or during cell culture.

5 **V. Therapeutic Uses**

[00111] In addition, one aspect of the invention provides for the therapeutic use of an agent that inhibits TC-PTP and/or stem cells that have been isolated or cultured using a method of the invention.

[00112] Abnormal signaling may affect the differentiating potential of
10 endothelial cells and their capacity to participate in the formation of new blood vessels, or repair of injured vessels. Early reperfusion of occluded coronary arteries reduces early mortality and improves the long-term prognosis of patients with an acute myocardial infarction. A number of clinical pilot trials have now shown that regeneration of myocardial function with autologous
15 intracoronary infusion of bone marrow-derived progenitor cells in patients after an acute myocardial infarction is feasible and safe (V. Schüchinger et al. *Nat Clin Pract Cardiovasc. Med.* 3:523-528, 2006). However, according to the latest REPAIR-AMI European clinical trial and the ASTAMI trial, the number of stem cells transplanted may predict success (V. Schüchinger et al. *N. Engl. J.*
20 *Med.* 355:1210-21, 2006; V. Schüchinger et al. *Eur. Heart J.* 27:2775-83, 2006; S. Erb et al. *Circulation.* 116:366-74, 2007; K Lunde et al. *Am. Heart J.* 154:710 e1-8, 2007). Thus augmenting the stem cell population is very attractive.

[00113] Accordingly, one embodiment provides a method of increasing
25 stem cells comprising administering an agent that inhibits TC-PTP to an animal in need thereof. Another embodiment provides use of an agent that inhibits TC-PTP for increasing stem cells in an animal in need thereof. Another embodiment provides use of an agent that inhibits TC-PTP for the manufacture of a medicament for increasing stem cells in an animal in need
30 thereof. The term "an animal in need thereof", as used herein, is any animal

- 36 -

that would benefit from receiving stem cells. In a particular embodiment the animal is human.

[00114] As stem cells promote vasculogenesis, stems cells can be used to treat diseases and disorders that comprise vessel damage. In addition, augmenting stem cells is useful in a patient with a hematopoietic disorder. Also, augmenting stems cells is useful in a bone marrow transplant recipient. Accordingly, one embodiment provides a method or use of an agent that inhibits TC-PTP for treating a vessel disease. Another embodiment provides a method or use of an agent that inhibits TC-PTP for treating a hematopoietic cell disorder. Another embodiment provides a method or use in an animal wherein the animal receives a bone marrow transplant. In certain embodiments the agent that inhibits TC-PTP is a small molecule inhibitor. In other the small molecule inhibitor is a thioxothiazolidinone compound. In yet further embodiments, the agent that inhibits TC-PTP is a RNAi sequence. In another embodiment agent that is a vanadate compound. In certain embodiments the agent is administered locally. In other embodiments the agent is administered systemically.

[00115] In one embodiment, an inhibitor of TC-PTP is administered to an animal. In another embodiment an inhibitor of TC-PTP is used on a patient's bone marrow-derived stem cells post-purification, prior to reinfusion, to augment the stem cell pool. In another embodiment an inhibitor of TC-PTP is used on a patient's bone marrow directly to expand stem cells and the resulting population of cells is injected into the patient.

[00116] Stem cells expanded or isolated using a method of the invention can be reinfused using methods known in the art including systemic reinfusion, percutaneous intra-coronary infusion, left ventricular catheter-based intramyocardial injection, surgical intramyocardial injection and intradermal reinfusion. Stem cells can also be injected into peripheral sites of ischemia such as in injured peripheral blood vessels.

[00117] Hematopoietic stem cell transplantation can be used to treat hematopoietic dyscrasias and malignancies (reviewed in Copelan EA. 2006.

- 37 -

Hematopoietic Stem-Cell Transplantation. *New Engl. J. Med.* 354:1813-1826), and in the treatment of solid organ malignancies, including renal cell carcinoma (Childs R, et al. 2000. Regression of Metastatic Renal-Cell Carcinoma after Nonmyeloablative Allogeneic Peripheral-Blood Stem-Cell Transplantation. *N. Engl. J. Med.* 343:750-75) and breast cancer (Stadtmauer EA, et al. 2000. Conventional-Dose Chemotherapy Compared with High-Dose Chemotherapy plus Autologous Hematopoietic Stem-Cell Transplantation for Metastatic Breast Cancer. *N. Engl. J. Med.* 342:1069-1076). Stem cells and agents that expand stem cells can be used for treating numerous genetic and degenerative disorders. Among them, age-related functional defects, hematopoietic and immune system disorders, heart failures, chronic liver injuries, diabetes, Parkinson's and Alzheimer's diseases, arthritis, and muscular, skin, lung, eye, and digestive disorders as well as aggressive and recurrent cancers could be successfully treated by stem cell-based therapies (Mimeault M et al, Clin Pharmacol Ther. 2007 Sep;82(3):252-64).

[00118] Bone marrow-derived stem cells, which also include marrow stromal cells (also termed mesenchymal stem cells), can be used for the repair of joint tissues such as articular cartilage, subchondral bone, menisci and tendons, thereby enhancing reparative signals in traumatic, degenerative and inflammatory joint disorders (De Bari C et al. *Clin Sci (Lond).* 113:339-48 2007). In addition, tissue-resident stem cells have been shown to contribute in muscle regeneration and repair, implying a role for these cells in aging and neuromuscular diseases (Musaro A et al. *Eur J Histochem.* 2007;51 Suppl 1:35-43). Enhanced revascularization of ischemic limbs, both in animal models and in clinical trials in humans has been documented.

[00119] Recent applications also include enhancing revascularization after myocardial ischemia, as well as neuroprotection after cerebral ischemia, among others (*Nat Clin Pract Cardiovasc Med.* 2006 Mar;3 Suppl 1:S23-8. See also S65, S69, S73 and S101). EPC can be also used to enhance endothelial cell repair after focal endothelial damage in atherosclerosis or diabetes as well as for other endothelial cell dysfunctions (*J Cell. Mol. Med.*

- 38 -

Vol 10, 2006, pp318-332) (Arterioscler. Thromb. Vasc. Biol. 2006;26;758-764). EPC can be used in cases of retinopathy and heart failure. EPC can also be used for valve tissue engineering (Circulation2006;114;132-137). The therapeutic methods of the invention can be used to treat any condition
5 wherein it is desirable to use stem cells.

[00120] Accordingly, one embodiment provides a method of enhancing revascularization comprising administering an agent that inhibits TC-PTP to an animal in need thereof. Another embodiment provides use of an agent that inhibits TC-PTP for enhancing revascularization. A further embodiment
10 provides use of an agent that inhibits TC-PTP for the manufacture of a medicament for enhancing revascularization. In certain embodiments, the ischemia is acute.

[00121] Revascularization is useful for treating ischemia such as results from myocardial infarction. Accordingly, one embodiment provides a method
15 of treating ischemia comprising administering an agent that inhibits TC-PTP to an animal in need thereof. Another embodiment provides use of an agent that inhibits TC-PTP for treating ischemia. A further embodiment provides use of an agent that inhibits TC-PTP for the manufacture of a medicament for treating ischemia.

[00122] Certain embodiments, provide a method of treating myocardial infarction comprising administering an agent that inhibits TC-PTP to an animal in need thereof. Another embodiment provides use of an agent that inhibits TC-PTP for treating myocardial infarction. A further embodiment provides use
20 of an agent that inhibits TC-PTP for the manufacture of a medicament for treating myocardial infarction.
25

[00123] As used herein, and as well understood in the art, "treating" is an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions,
30 diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease

- 39 -

progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treating" can also mean prolonging survival as compared to expected survival if not receiving treatment.

5 **VI. Compositions**

[00124] The present invention also includes pharmaceutical compositions containing an agent that inhibits TC-PTP for use in the methods of the invention. Accordingly, the present invention provides a pharmaceutical composition for expanding stem cells comprising an effective amount of an
10 agent that inhibits TC-PTP in admixture with a suitable diluent or carrier.

[00125] Compositions containing compounds of Formula I can contain pure enantiomers or pure diastereomers or mixtures of enantiomers, for example in the form of racemates, or mixtures of diastereomers. Mixtures of two or more stereoisomers of compounds are further contemplated with
15 varying ratios of stereoisomers in the mixtures. Compositions of compounds of Formula I can also contain *trans*- or *cis*-isomers including pure *cis*-isomers, pure *trans*-isomers or *cis/trans*-isomer mixtures with varying ratios of each isomer. When a composition containing a pure compound is desired, diastereomers (*e.g.*, *cis/trans*-isomers) can be separated into the individual
20 isomers (*e.g.*, by chromatography) or racemates (*e.g.*, separated using standard methods such as chromatography on chiral phases or resolution by crystallization of diastereomeric salts obtained with optically active acids or bases). Stereochemically uniform compositions of compounds identified herein can also be obtained by employing stereochemically uniform reactants
25 or by using stereoselective reactions.

[00126] Therapeutic or prophylactic compositions are administered to a subject in amounts sufficient augment stem cell numbers. In one embodiment, the compositions are administered to augment EPC. The effective amount can vary according to a variety of factors such as the subject's condition,
30 weight, sex and age. Other factors include the mode of administration. The appropriate amount can be determined by a skilled physician. In general, an

- 40 -

effective amount is one which alleviates one or more signs or symptoms of the disease or condition being controlled or treated.

[00127] Compositions can be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents
5 may be desirable.

[00128] The compositions can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compositions can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release
10 formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they can also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well-known to those of ordinary skill in the pharmaceutical arts.

15 **[00129]** Advantageously, compositions can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three or four times daily for example. Furthermore, compositions can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well-
20 known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

[00130] The dosage regimen utilizing the compositions is selected in accordance with a variety of factors including type, species, age, weight, sex
25 and medical condition of the subject; the severity of the condition to be treated; the route of administration; the renal, hepatic and cardiovascular function of the subject; and the particular composition thereof employed. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the composition required to prevent, counter or arrest
30 the progress of the condition. Optimal precision in achieving concentrations of composition within the range that yields efficacy without toxicity requires a

- 41 -

regimen based on the kinetics of the composition's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a composition.

[00131] Such pharmaceutical compositions can be for intralesional, intravenous, topical, rectal, parenteral, local, inhalant or subcutaneous, intradermal, intramuscular, intrathecal, transperitoneal, oral, and intracerebral use. The composition can be in liquid, solid or semisolid form, for example pills, tablets, creams, gelatin capsules, capsules, suppositories, soft gelatin capsules, gels, membranes, tubelets, solutions or suspensions.

[00132] The present invention also provides pharmaceutical compositions comprising stem cells. The stem cells may be endothelial progenitor cells or hematopoietic stem cells.

[00133] The pharmaceutical compositions of the invention can be intended for administration to humans or animals. Dosages to be administered depend on individual needs, on the desired effect and on the chosen route of administration.

[00134] The pharmaceutical compositions can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to patients, and such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in *Remington's Pharmaceutical Sciences* (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985).

[00135] On this basis, the pharmaceutical compositions include, albeit not exclusively, the active compound or substance in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids. The pharmaceutical compositions may additionally contain other

- 42 -

agents such as other agents that can prevent the inhibition of apoptosis or that are used in treating inflammatory conditions or sepsis.

[00136] The following non-limiting examples are illustrative of the present invention:

5 **EXAMPLES**

Example 1

Materials and Methods

Mice

[00137] Generation of TC-PTP and PTP1B mutant mice was described
10 previously ². Experiments were performed with mice on a mixed Balb/c–
129SJ background and with mice backcrossed for 8 generation on Balb/c. All
procedures were approved by the McGill University Research and Ethics
Animal Committee, and experiments were carried out according to the
Canadian Council on Animal Care ethical regulations.

15 **Flow cytometry (murine cells)**

[00138] Bone marrow cell suspensions were prepared from tibia, femur,
humerus and ulna of TC-PTP^{+/+} and TC-PTP^{-/-} mice (age 14-19 d) or only
from femurs of adult Balb/c mice in PBS containing 2% FBS, and filtered
through a 70 µm cell strainer. Mononuclear cells were also obtained from
20 peripheral blood and were isolated using “Lympholyte” (Cedarlane, Hornby,
Ontario, Canada) according to the manufacturer's instructions and
resuspended in PBS containing 2% FBS. All types of cell suspensions were
incubated with purified anti-CD16/CD32 mAb (BD Biosciences, Mississauga,
ON, Canada) to block nonspecific binding to Fcγ receptors, followed by a
25 combination of the indicated antibodies. Reactions were incubated at 4°C for
30 min, in 100 µl PBS containing 2% FBS, followed by washing in the same
solution. When appropriate, cells were then stained with streptavidin-Pacific
Blue at 4°C for 20 min, in 100 µl PBS containing 2% FBS, followed by
washing in the same solution. Data acquisition was performed using a
30 FACScan or FACSaria flow cytometer (BD Biosciences), and analysis was

- 43 -

done with CellQuest Pro software (BD Biosciences) and FloJo software. Antibody-fluorochrome conjugates used were as follows: CD14-APC (Sa2-8; eBioscience, San Diego, CA), CD105-biotin (MJ7/18; eBioscience), CD117-PECy5 (2B8; Biolegend, Vineland, ON, Canada), Sca-1-FITC or Sca-1 PECy7 (D7; BD Biosciences), streptavidin-Pacific Blue (Molecular Probes, Burlington, ON, Canada). Lineage (Lin) markers comprised the following combination of antibody-PE conjugates: CD3 ϵ (145-2C11; BD Biosciences), CD4 (RM4-5; BD Biosciences), CD5 (53-7.3; Biolegend), CD8 (53-6.7; BD Biosciences), CD11b (M1-70; BD Biosciences), CD19 (1D3; BD Biosciences), CD31 (MEC 13.3; BD Biosciences), CD49b (9C10; Biolegend), CD127 (SB/199; BD Biosciences), CD135 (A2F10; eBioscience), Ter-119 (BD Biosciences).

Flow cytometry (human cells)

[00139] Bone marrow cells from healthy donors were obtained from Stem Cell Technologies. Cell suspensions were prepared in PBS containing 2% FBS, and filtered through a 70 μ m cell strainer. Cell suspensions were then stained with the indicated antibodies. Reactions were incubated at 4°C for 30 min, in 100 μ l PBS containing 2% FBS, followed by washing in the same solution. When appropriate, cells were then stained with streptavidin-Pacific Blue at 4°C for 20 min, in 100 μ l PBS containing 2% FBS, followed by washing in the same solution. Data acquisition was performed using a FACSAria or FACS LSRII flow cytometer (BD Biosciences), and analysis was done with FloJo software (BD Biosciences). Antibody-fluorochrome conjugates used were as follows: CD14-PECy7 (M5E2; Biolegend), CD34-FITC (AC136; Miltenyi Biotec; Auburn, CA), CD105-biotin (166707; Cedarlane Laboratories; Horby, ON, Canada), CD117-PECy5 (A3C6E2; Biolegend), CD133-APC (AC133; Miltenyi Biotec), streptavidin-Pacific Blue (Molecular Probes, Burlington, ON, Canada). Lineage (Lin) markers comprised the following combination of antibody-PE conjugates: CD3 (UCHT1; Biolegend), CD4 (RPA-T4; Biolegend), CD5 (UCHT2; Biolegend), CD8 (RPA-T8; Biolegend), CD11b (ICRF44; Biolegend), CD19 (HIB19; Biolegend), CD31

- 44 -

(WM59; Biolegend), CD127 (HCD127; Biolegend), CD135 (BV10A4H2; Biolegend), CD235ab (HIR2; Biolegend).).

Phosphoflow

5 [00140] Intracellular staining was performed post-surface staining of the cells. Cells were fixed and permeabilized with CytoPerm CytoFix according to the to the manufacturer's instructions (BD Biosciences). The phospho-CD117 antibody was obtained from Cell Signaling Technology (Danvers, MA), the non-specific IgG from BD Biosciences, and the anti-rabbit Alexa 488 conjugate from Invitrogen-Molecular Probes (Burlington, ON, Canada).

10 **Endothelial progenitor cell colony-forming assay**

[00141] Whole bone marrow was obtained from TC-PTP^{+/+} and TC-PTP^{-/-} mice aged 14 to 19 d. Cells, 5×10^6 , were plated for 48 h on 6-well plates coated with $5 \mu\text{g}/\text{cm}^2$ fibronectin in 2 ml EndoCult culture medium (StemCell Technologies, Vancouver, BC, Canada). Nonadherent cells were harvested
15 by collecting culture supernatant, and counted manually. Cells, 5×10^5 , were replated for 72 h on 24-well plates coated with fibronectin in 1 ml EndoCult culture medium. Colonies were counted using an inverted microscope at 40X magnification. For colony assays using purified stem cells, whole bone marrow was harvested as above, and flow cytometry was used to isolate the
20 desired cell fractions. Using a FACSaria flow cytometer, a 2-way sort was performed to collect 400 cells into 0.5 ml EndoCult culture medium. Cells were then transferred into 48-well plates coated with fibronectin for 3 to 5 d. Colonies were counted as above.

Sodium orthovanadate treatment of bone marrow cells

25 [00142] Whole bone marrow was obtained from TC-PTP^{+/+} and TC-PTP^{-/-} mice aged 14 to 19 d, and from adult Balb/c mice. Cells, 4×10^5 , were plated in 6-well plates in 2 ml Iscove's modified Dulbecco's medium (Stem Cell Technologies) supplemented with 10% FBS and 0.02% v/v β -mercaptoethanol for 24 h or 48 h. Where indicated, sodium orthovanadate
30 (Sigma-Aldrich, Oakville, ON, Canada) was added at the start of culture to a

- 45 -

final concentration of 2 μ M, 10 μ M or 50 μ M. After incubation, cells were harvested and analyzed by flow cytometry.

Statistical analysis

[00143] Values are reported as the mean $\pm$ SEM of at least 3
5 independent experiments. The statistical significance of differences between groups was assessed using a two-tailed, unpaired Student's *t* test.

Results

Characterization of bone marrow-derived stem cells in TC-PTP mice

[00144] The inventors have previously demonstrated multiple
10 hematopoietic defects in the bone marrow of TC-PTP^{-/-} mice, suggesting a primary anomaly of HSC. To further characterize this population, bone marrow was obtained from TC-PTP^{+/+} and TC-PTP^{-/-} mice. Cells were then analyzed by flow cytometry after staining for surface expression of CD117 and Sca-1, which are accepted markers of HSC. Typical Lin markers were used to
15 exclude mature progenitor cells. In addition, CD31, CD127 and CD135 were used to exclude mature endothelial cells (EC), common lymphoid progenitors (CLP), and short-term HSC, respectively (Figure 1). CD105 is generally considered a marker of endothelial cells, but is also expressed on a subset of circulating CD34⁺ human hematopoietic progenitors; its expression on bone
20 marrow HSC has not been characterized.

[00145] Pluripotent stem cells were defined as Lin⁻CD117⁺. This subpopulation was further subdivided based on surface expression of Sca-1 and CD105 (Figure 2A). In wild type marrow, two distinct populations could be identified: Sca-1⁺CD105⁻ (I) and Sca-1⁺CD105⁺ (II). However, in TC-PTP^{-/-}
25 bone marrow, two additional populations were observed: Sca-1^{hi}CD105⁻ (III) and Sca-1^{hi}CD105⁺ (IV). In addition, there was a distinct increase in the percentage of Sca-1^{+/hi}CD105⁺ cells in TC-PTP^{-/-} bone marrow compared to control. Comparison of absolute cell counts revealed a 5-fold increase in the number of Lin⁻CD117⁺Sca-1^{+/hi} cells in TC-PTP^{-/-} bone marrow compared to
30 control (Figure 2B). Fractionation of this population according to CD105

- 46 -

expression revealed a 9-fold increase in the number of Lin⁻CD117⁺Sca-1^{+/hi}CD105⁺ cells in TC⁻PTP^{-/-} bone marrow and no significant difference in the number of Lin⁻CD117⁺Sca-1^{+/hi}CD105⁻ cells compared to control.

[00146] Figure 2 shows increased numbers of EPC in TC-PTP^{-/-} bone marrow. In Figure 2A, TC-PTP^{+/+} and TC-PTP^{-/-} bone marrow cells were stained with antibodies to lineage (Lin) markers, and to CD105, CD117 and Sca-1, and analyzed by flow cytometry as described in *Materials and Methods*. The absolute cell count for the Lin⁻CD117⁺ population per 1 X 10⁶ bone marrow cells is indicated (*left panels*). The Lin⁻CD117⁺Sca-1⁺ population was further fractionated into 4 subsets (I-IV) based on the level of expression of Sca-1 (low or high), and on surface expression of CD105; the percentage of cells in each subset is indicated (*right panels*). (B) Absolute cell counts (per 1 X 10⁶ bone marrow cells) for the total Lin⁻CD117⁺ population and its CD105⁻ and CD105⁺ subsets were obtained by flow cytometry. P < 0.005.

15

Example 2

[00147] Hematopoietic and endothelial cell lineages share a common precursor. Because long-term HSC and endothelial progenitor cells (EPC) are indistinguishable based on currently known cell surface markers (Figure 1), the number of EPC within the Lin⁻CD117⁺Sca-1^{+/hi}CD105⁻ and Lin⁻CD117⁺Sca-1^{+/hi}CD105⁺ pluripotent stem cell subsets was quantified using an endothelial cell colony (CFU-EC) assay (Figure 2C). When whole bone marrow was assayed, a 5-fold increase was observed in the number of CFU-EC in TC⁻PTP^{-/-} bone marrow compared to control, whereas a 7-fold increase in CFU-EC was seen with purified Lin⁻CD117⁺Sca-1^{+/hi}CD105⁺ cells. Few endothelial colonies were seen with Lin⁻CD117⁺Sca-1^{+/hi}CD105⁻ cells.

[00148] To confirm the cellular composition of the colonies generated, cells from these CFU-EC were extracted and analyzed by flow cytometry (Figure 2D). All cells from TC⁻PTP^{+/+} and TC⁻PTP^{-/-} mice expressed known mature endothelial markers: CD31 (PECAM-1), Tie-2, Flk-1 and CD105.

30

- 47 -

Together, these results indicate that the number of EPC is increased in the bone marrow of TC-PTP^{-/-} mice. These EPC are characterized by a Lin⁻CD117⁺Sca-1^{+hi}CD105⁺ phenotype and can differentiate into mature endothelial cells when cultured under appropriate conditions.

- 5 **[00149]** EPC give rise to circulating EPC (CEPC) within the bone marrow, which are characterized by surface expression of CD14, in addition to EPC markers (Figure 1). Therefore, the number of CD14⁺ cells within the Lin⁻CD117⁺Sca-1^{+hi}CD105⁺ subpopulation was assessed by flow cytometry to quantify the number of CEPC (Figure 3A). Comparison of the relative
10 number of CD14⁺ cells revealed a 50% increase in CEPC in TC-PTP^{-/-} bone marrow compared to control. Thus, both the number of EPC and CEPC is increased in the bone marrow of TC-PTP^{-/-} mice, and the EPC are characterized by a Lin⁻CD117⁺Sca-1^{+hi}CD105⁺ phenotype.

Fate of the stem cells in the periphery

- 15 **[00150]** Both EPC and CEPC can circulate in peripheral blood. Although found in smaller numbers than in the bone marrow, these cells can be identified with the same surface markers. Flow cytometry analysis of peripheral blood revealed an augmentation of this rare EPC population (Figure 3B). To identify CEPC, the EPC subpopulation was further subdivided
20 based on surface expression of CD14 and CD105. An average 5-fold increase in CEPC was noted by flow cytometry (Figure 3B). The endothelial potential of these peripheral blood progenitors was confirmed by placing purified peripheral blood leukocytes in an endothelial cell colony assay (CFU-EC) (Figure 3C). Using this method, a 3.6-fold increase was observed in the
25 number of CFU-EC in TC-PTP^{-/-} blood compared to control. Thus, the increased number of bone marrow EPC and CEPC in TC-PTP^{-/-} mice is reflected by a corresponding increase in their number in peripheral blood, indicating that these stem cells can be mobilized from the bone marrow and can circulate in the entire organism.

- 30 ***The c-kit pathway is hyperactivated in TC-PTP^{-/-} stem cells***

- 48 -

[00151] To explore the consequences of the lack of TC-PTP on signaling receptors and molecules important for the biology of stem cells, the phosphorylation pattern of key receptor pathways involved in stem cell maintenance and proliferation was investigated. Because of the low frequency
5 of bone marrow stem cells (~1/500 in a normal Balb/c mouse), the phosphorylation pattern in stem cell subpopulations was studied by flow cytometry using phosphoflow. Intracellular staining of bone marrow Lin⁻ CD117⁺Sca-1^{+/hi} EPC with fluorescent Ab to phosphorylated c-kit (CD117) or total CD117 protein was followed by flow cytometry analysis for both TC⁻
10 PTP^{+/+} and TC⁻PTP^{-/-} mice; each staining reaction also contained fluorescent control IgG to control for nonspecific staining of intracellular proteins (Figure 4). Under quiescent conditions, CD117 in TC⁻PTP^{+/+} EPC is not phosphorylated, as demonstrated by equivalent mean fluorescent intensity (MFI) of phosphorylated CD117 and IgG control (MFI 30 v. 29). However, TC-
15 PTP^{-/-} EPC showed constitutive phosphorylation of CD117, as demonstrated by a 2-fold increase in MFI compared to IgG control (MFI 48 v. 24), and a 1.6-fold increase compared to TC-PTP^{+/+} EPC (MFI 48 v. 30). This differential phosphorylation pattern is significant, as equivalent amounts of total CD117 protein were detected in TC-PTP^{+/+} and TC-PTP^{-/-} EPC (Figure 4A). The
20 average MFI obtained from several Phosflow experiments are presented in Figure 4B. Normalizing the MFI obtained for total CD117 protein to that obtained for control IgG demonstrates equal expression of CD117 in TC-PTP^{+/+} and TC-PTP^{-/-} EPC. In contrast, comparing phosphorylated CD117 to total CD117 protein reveals an almost 2-fold increase in the
25 phosphorylation level of CD117 in TC-PTP^{-/-} cells versus wild type controls. Similarly, CD117 in TC⁻PTP^{+/+} bone marrow CEPC is unphosphorylated under unstimulated conditions, whereas TC-PTP^{-/-} EPC show constitutive phosphorylation of CD117, as demonstrated by a 1.8-fold increased in MFI compared to IgG control (MFI 55 v. 30), and a 1.5-fold increase compared to
30 TC⁻PTP^{+/+} EPC (MFI 55 v. 36) (Figure 4C). The average MFI obtained from several Phosflow experiments are presented in Figure 4D. In addition to confirming hyperphosphorylation of CD117 in TC-PTP^{-/-} CEPC, an increased

- 49 -

phosphorylation was noted that was even more pronounced in CEPC compared to EPC, as unequal amounts of CD117 protein were detected in TC-PTP^{+/+} and TC-PTP^{-/-} CEPC. Indeed, levels of CD117 protein are consistently 1.8-fold lower in TC-PTP^{-/-} CEPC compared to wild type controls (average MFI 438 v. 250). Thus, in the absence of TC-PTP, a known negative regulator of several cytokine signaling pathways, hyperphosphorylation of CD117 was observed in bone marrow EPC and CEPC, suggesting an effect of TC-PTP enzymatic activity on this pathway. Concurrently, levels of CD117 were significantly decreased in TC-PTP^{-/-} CEPC, and to a lesser degree in EPC, suggesting an additional indirect effect of TC-PTP in regulating expression of CD117.

Example 3

[00152] To determine whether the increase in stem cell numbers was attributable to lack of TC-PTP enzymatic activity and not simply due to absence of TC-PTP protein, orthovanadate, a nonspecific inhibitor of protein tyrosine phosphatases was employed. Whole bone marrow was obtained from adult Balb/c mice, and cultured in the presence of increasing concentrations of orthovanadate (Figure 5). Cells were harvested at the indicated time points, and analyzed by flow cytometry to determine the number of pluripotent stem cells, identified as Lin⁻CD117⁺Sca-1⁺. Comparison of absolute cell counts (number indicated in each plot) revealed a 1.8-fold increase in the number of pluripotent stem cells after 24 h of treatment with 50 μM orthovanadate, whereas no difference was observed using lower concentrations of inhibitor. In contrast, after 48 h of culture, each successive increase in vanadate concentration resulted in a corresponding increase in the number of pluripotent stem cells, reaching a maximum 9-fold increase when comparing culture in 50 μM orthovanadate to control. Calculating the ratio between the number of pluripotent stem cells at 24 h and 48 h reveals a 5-fold increase in the rate of proliferation between cultures supplemented with 50 μM orthovanadate and control cultures. Together, these results indicate

- 50 -

that TC-PTP, and potentially other protein tyrosine phosphatases, are implicated in the regulation of hematopoietic and endothelial stem cell proliferation, and support the notion that pharmacological inhibitors of these enzymes may be employed to augment the stem cell population, with obvious
5 clinical implications.

[00153] In Figure 5 wild type Balb/c bone marrow cells were cultured in the presence of indicated concentrations of sodium orthovanadate. Cultures were analyzed by flow cytometry 24 h and 48 h post-treatment. Cells were stained with antibodies to lineage (Lin) markers, and to CD117 and Sca-1, as
10 described in *Materials and Methods*. The absolute cell count (per 1×10^6 marrow cells) for the CD117⁺Sca-1⁺ fraction is indicated.

Example 4

Effects of TC-PTP blocking agents

15 *RNAi treatment of bone marrow cells*

Materials and Methods

[00154] Applicable materials and methods were used as described in Example 1.

[00155] Murine or human bone marrow cells (5×10^6), were
20 electroporated (320 V, 960 μ F) with 1 μ M of TC-PTP specific RNAi sequence (TC1: sense: GCCCAUAUGAUCACAGUCG (SEQ ID NO: 1), exon 2, human and mouse; Ambion Austin, TX)) (TC2: sense: GGCACAAAGAAGUUACAUC (SEQ ID NO: 2), exon 3, mouse only; Ambion Austin, TX) or scramble RNAi sequence (SCR). Cells were let to recover and were plated in 6-well plates
25 coated with fibronectin in EndoCult media (Stem Cell Technologies) for 48 h. After incubation, cells were harvested and analyzed by flow cytometry.

Results

[00156] To determine whether the increase in stem cell numbers observed in TC-PTP^{-/-} mice was attributable to lack of TC-PTP enzymatic

- 51 -

activity and not simply due to absence of TC-PTP protein, we developed efficient TC-PTP blocking agents and tested their capacity to expand the bone marrow EPC subpopulation.

[00157] Expression of TC-PTP in bone marrow cells was suppressed
5 using RNA interference (RNAi). RNAi sequences are molecules known to degrade in a specific fashion their target RNA, and they have been reported to successfully achieve TC-PTP inhibition ³⁸. Two TC-PTP-specific RNAi sequences were developed and demonstrated increased efficiency by using them in combination. Whole bone marrow was obtained from adult Balb/c
10 mice and from control TC-PTP^{+/-} and PTP1B^{-/-} animals. TC-PTP-specific RNAi sequences as well as a control scramble sequence (SCR) were delivered to bone marrow progenitors by electroporation ³⁹. On average, at least 80% of stem cells take up the RNA with this method. After electroporation, cells were cultured for 48 h, and the functional effects of TC-PTP-specific inhibition was
15 monitored by counting the absolute number of Lin⁻CD117⁺Sca-1^{+/hi} EPC by flow cytometry (Figure 6). Comparison of absolute EPC counts from Balb/c mice revealed a similar number of EPC in PBS and SCR conditions. However, a significant increase in the number of EPC was noted after 48 h of treatment with TC-PTP-specific RNAi sequence (Figure 6A). The effect of RNAi
20 treatment was also observed in bone marrow EPC from TC-PTP^{+/-} mice, although the relative increase was not as striking due to a higher intrinsic number of EPC in these mice (Figure 6A). Since TC-PTP shares great homology with PTP1B, the possibility that this phosphatase might also be implicated was examined by studying the effects of the RNAi sequences on
25 cells lacking this enzyme. To this end, the experiments were repeated using bone marrow from PTP1B^{-/-} mice. Untreated PTP1B^{-/-} marrow contained similar numbers of EPC compared to Balb/c marrow. Treatment of PTP1B^{-/-} marrow cells with TC-PTP-specific RNAi sequences also resulted in increased numbers of stem cells (Figure 6A). The average absolute stem cells counts
30 obtained from several experiments are presented in Figure 6B. Electroporation of TC-PTP-specific RNAi sequences in Balb/c bone marrow cells produced a 3.1-fold increase in the number of Lin⁻CD117⁺Sca-1^{+/hi} EPC

- 52 -

compared to PBS and SCR controls ($p < 0.01$). Using this method to further decrease the expression of TC-PTP in TC-PTP^{+/-} bone marrow cells only achieved a 1.3-fold increase in the stem cell subpopulation compared to PBS and SCR controls ($p = \text{NS}$); however, TC-PTP^{+/-} bone marrow contains about
5 four times as many EPC compared to wild type marrow, masking the effect of treatment with RNAi. A significant augmentation in the numbers of EPC (2.7-fold) was observed when treating PTP1B^{-/-} bone marrow cells with TC-PTP-specific RNAi sequences compared to PBS and SCR controls ($p < 0.05$). To ensure that treatment with RNAi sequences did not affect the ability of stem
10 cells to differentiate into endothelial cells, an endothelial cell colony assay was performed (CFU-EC) (Figure 6C). Balb/c bone marrow, untreated (PBS) or electroporated with SCR or TC-PTP-specific RNAi sequences was assayed. A 2.4-fold increase was observed in the number of CFU-EC using RNAi-treated stem cells compared to PBS control ($p < 0.01$). These results describe a novel
15 and simple method to increase the bone marrow EPC subpopulation *in vitro*: using TC-PTP-specific RNAi sequences to inhibit the *de novo* expression of TC-PTP, a 2 to 3-fold increase in the number of functional bone marrow EPC can be achieved.

20 Example 5

Effects of TC-PTP blocking agents

Small molecule inhibitor treatment of bone marrow cells

Materials and Methods

[00158] Applicable materials and methods were used as described in
25 Example 1.

[00159] Murine or human bone marrow cells (5×10^6), were plated in 6-well plates coated with fibronectin in EndoCult media (Stem Cell Technologies) for 48 h. Where indicated, a small molecule inhibitor previously described⁴⁰, was added at the start of culture to a final concentration of 10 μM

- 53 -

or 50 μ M. After incubation, cells were harvested and analyzed by flow cytometry.

Results

[00160] Small chemical inhibitors of protein tyrosine phosphatases have potential applications in the treatment of several human diseases and possess the advantage of direct uptake by target cells. The inventors have identified a unique uncharged thioxothiazolidinone derivative, which is capable of inhibiting PTP1B and TC-PTP. This small molecule can penetrate cells, inhibit the catalytic pocket of these two enzymes, and cause hyperphosphorylation of a known substrate of PTP1B and TC-PTP⁴⁰. Since the absence of PTP1B does not affect bone marrow progenitor cell numbers, the inventors reasoned that this compound could have great potential in this system. Whole bone marrow was obtained from adult Balb/c mice and from control TC-PTP^{+/-} and PTP1B^{-/-} animals, and cultured in the presence of increasing concentrations of the inhibitor (Figure 6D). Cells were harvested at 48 h, and analyzed by flow cytometry to determine the number of EPC, identified by a Lin⁻CD117⁺Sca-1⁺ phenotype. Comparison of absolute cell counts showed that each successive increase in inhibitor concentration resulted in a corresponding increase in the number of stem cells, reaching significance at 50 μ M, compared to DMSO control (p=0.02). Likewise, treatment of TC-PTP^{+/-} and PTP1B^{-/-} bone marrow cells with the inhibitor resulted in an increased number of stem cells (Figure 6D). The average absolute stem cells counts obtained from several experiments are presented in Figure 6E. Treatment of Balb/c bone marrow cells with inhibitor at a concentration of 50 μ M achieved a 3.4-fold increase in the number of Lin⁻CD117⁺Sca-1^{+/hi} EPC compared to DMSO control (p=0.02). Using this method to further decrease the expression of TC-PTP in TC-PTP^{+/-} bone marrow cells achieved a smaller (1.5-fold) but significant (p=0.04) increase in the stem cell subpopulation compared to DMSO control. A significant augmentation in the numbers of EPC (5.1-fold) was also observed when treating PTP1B^{-/-} bone marrow cells under similar conditions (p<0.05). Again, we confirmed the

- 54 -

potential of the augmented stem cell populations to differentiate into endothelial cells using an endothelial cell colony assay (CFU-EC) (Figure 6F). Balb/c bone marrow, untreated (DMSO) or cultured with inhibitor at concentrations of 10 μ M or 50 μ M, was assayed. A 4-fold increase in the number of CFU-EC was observed using progenitor cells treated with inhibitor (50 μ M) compared to DMSO control ($p < 0.001$).

Example 6

Clinical application in humans

[00161] To bridge the above findings from the mouse to the human setting, it is necessary to correlate murine markers with their human homologs. The human antibodies and markers corresponding to those used in the mouse have been identified. Table 1 provides a list of the 15 murine markers used for the characterization of EPC and CEPC in mice and indicates, when available, a human homolog. Murine markers used to isolate bone marrow stem cell populations are listed with the international CD nomenclature along with other commonly used names for those antigens. Of the 15 markers, only Sca-1 and Ter-119 do not have a human counterpart. Sca-1 is a marker of stem cells in mice only; however, other surface antigens such as CD133 and CD34 can replace this marker in humans. Ter-119 is used to exclude erythroid cells; in humans, CD235a can be used to replace this marker. Accordingly, human EPC were defined as Lin⁻ CD34⁺CD133⁺CD117⁺. This subpopulation was further subdivided based on surface expression of CD105 and CD14 to identify CEPC.

Table 1. Selected CD nomenclature and expression pattern. The existence of a human homolog corresponding to each surface marker is indicated.

Murine Antigen	Other names	Human Homolog
CD3 ϵ	T3 complex	Yes
CD4	Ly4, L3T4	Yes

- 55 -

CD5	Ly1	Yes
CD8	Ly2/Ly3 (α/β subunits)	Yes
CD11b	Mac-1, Ly40, CR3 integrin αM	Yes
CD14	LPS-R, Mo2	Yes
CD19	B4	Yes
CD31	PECAM-1, gpIIa	Yes
CD49b	DX5, VLA-2, Integrin α=2	Yes
CD105	Endoglin, Eng	Yes
CD117	c-kit, SCFR, Steel factor R	Yes
CD127	IL-7Rα	Yes
CD135	Flt3/Flk2, Ly72	Yes
Sca-1	Ly-6A/E	No Replaced by CD133 & CD34
Ter-119	Ly76	No Replaced by CD235a

[00162] Of the two TC-PTP specific-RNAi sequences that were developed for the murine studies, only one reacted with human TC-PTP. TC1 (GCCCAUAUGAUCACAGUCG SEQ ID NO: 1) TC-PTP-specific RNAi sequence as well as a control scramble sequence (SCR) were delivered to human bone marrow progenitors by electroporation³⁹. After electroporation, cells were cultured for 48 h and the functional effects of this TC-PTP-specific inhibition was monitored by counting the absolute number of Lin⁻ CD34⁺CD133⁺CD117⁺ EPC and Lin⁻CD34⁺CD133⁺CD117⁺CD105⁺CD14⁺ CEPC by flow cytometry (Figure 7). Comparison of absolute EPC and CEPC counts from human bone marrow revealed a similar number of EPC and CEPC in PBS and SCR conditions. In contrast, a significant increase in the size of these populations was observed after 48 h of treatment with TC-PTP-specific RNAi sequence (Figure 7A). The average absolute stem cells counts obtained from several experiments are shown in Figure 7B. Electroporation of TC-PTP-specific RNAi sequence in human bone marrow cells produced a 3.2-

- 56 -

fold increase in the number of Lin⁻CD34⁺CD133⁺CD117⁺ EPC compared to PBS and SCR controls (p=0.05), and a 5.3-fold increase in the number of Lin⁻CD34⁺CD133⁺CD117⁺CD105⁺CD14⁺ CEPC relative to controls (p<0.05). These results correlate with the *in vitro* mouse model and demonstrate the feasibility of decreasing TC-PTP expression to expand the population of EPC and CEPC in humans.

[00163] The efficacy of the small chemical inhibitor of TC-PTP and PTP1B was also assessed in a human system. Human bone marrow was cultured in the presence of increasing concentrations of inhibitor (Figure 7C). Cells were harvested at 48 h, and analyzed by flow cytometry to determine the number of EPC (Lin⁻CD34⁺CD133⁺CD117⁺) and CEPC (Lin⁻CD34⁺CD133⁺CD117⁺CD105⁺CD14⁺). Comparison of absolute cell counts showed a significant increase in the number of stem cells at the lower concentration of inhibitor (10 μ M). There was no additional increase in the number of EPC and CEPC using a concentration of 50 μ M (Figure 7C). The average absolute stem cells counts obtained from several experiments are shown in Figure 7D. Treatment of human bone marrow cells with inhibitor at a concentration of 10 μ M, resulted in a 2.8-fold increase in the number of Lin⁻CD34⁺CD133⁺CD117⁺ EPC compared to DMSO control (p<0.05), and a 3.6-fold increase in the number of Lin⁻CD34⁺CD133⁺CD117⁺CD105⁺CD14⁺ CEPC relative to DMSO control (p<0.05). Together, these results confirm that EPC and CEPC populations can be increased after treatment of human bone marrow stem cells with a TC-PTP blocking agent, and support the use of such pharmacological inhibitors in the clinical setting.

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Example 7

Animal models

[00164] Endothelial progenitor cells (EPCs) are known to promote repair of the cardiovascular system after injury. The inventors have demonstrated that TC-PTP deficient mice have an increased EPC and CEPC in the bone

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- 57 -

marrow as well as in the peripheral blood. Progenitor cells from TC-PTP^{-/-} mice or normal stem cells treated with TC-PTP blocking agents are used to promote repair of blood vessels or ischemic heart after injury in a mouse model.

- 5 **[00165]** Animals are first subjected to femoral artery ligation or left anterior descending (LAD) coronary artery ligation and then treated with stem cells.

Hindlimb ischemia (femoral artery ligation)

- 10 **[00166]** Revascularization in a hindlimb ischemia model (ligation of the femoral artery) and recovery is accomplished using bone marrow stem cell transplant or administration of an agent that inhibits TC-PTP.

- 15 **[00167]** Under general anesthesia, the right femoral artery is exposed using a longitudinal skin incision of < 1 cm, and ligated with 6-0 silk, just distal to the profunda femoris branch, and proximal to the genus descendens artery. The muscles and skin are closed layer by layer with 6-0 absorbable and nylon sutures, respectively. The contralateral leg is used as the unoperated control. Mice are placed under a heat lamp to recover.

Myocardial infarction (left anterior descending [LAD] coronary artery ligation)

- 20 **[00168]** Repair of ischemic heart in a myocardial infarction model (left anterior descending [LAD] coronary artery ligation) is accomplished using bone marrow stem cell transplant or administration of TC-PTP blocking agent.

- 25 **[00169]** This operation is performed under general anesthesia and the mouse is ventilated artificially with a respirator. An oblique 8 mm incision is made 2 mm away from the left sternal border, towards the left armpit. The muscles are separated. The rib cage and moving left lung are then visualized. The 4th intercostal space is then opened taking caution not to damage the lung. The chest retractor is inserted and opened gently to spread the wound 8-10 mm in width. The pericardium is gently picked up with curved and
30 straight forceps, pulled apart and placed behind the arms of the retractor. The

- 58 -

LAD artery is ligated 1-2 mm below the tip of the left auricle in its normal position, which induces roughly 40-50% ischemia of the left ventricle. A 7-0 silk ligature on a tapered needle is passed underneath the LAD coronary artery. The ligature is then tied with three knots. A syringe fitted with a 30 ga
5 needle is then used to inject 0.1 ml cell suspension in the border zone of the infarct. The retractor is removed and the lungs are reinflated by shutting off the ventilator outflow for 1-2 seconds. The chest cavity is closed by bringing together the 4th and the 5th ribs with one or two 6-0 nylon sutures (with pressure applied to the chest wall to reduce the volume of free air). The
10 muscles and skin are closed layer by layer with 6-0 absorbable and nylon sutures, respectively.

Treatments to increase stem cell number

[00170] Test animals are administered either: 1) genetically modified stem cells (using TC-PTP^{-/-} bone marrow), 2) ex-vivo culture enhanced EPC
15 and CEPC with TC-PTP blocking agent, 3) local administration of TC-P TP blocking agent or 4) systemic administration of TC-PTP blocking agent. Control animals are injected with a suitable carrier and/or TC-PTP^{+/+} stem cells.

Genetically modified stem cells

20 **[00171]** Operated mice are injected with bone marrow as a source of stem cells from TC-PTP^{-/-} mice one day post-surgery. Bone marrow cell suspension are injected i.v. (tail vein; 1 x 10E6 cells in PBS). The control mice are injected with PBS or TC-PTP^{+/+} bone marrow cells.

[00172] Revascularization and repair of ischemic heart is assessed by
25 comparing the degree of recovery from the induced ischemic damage in operated animals treated with TC-PTP deficient cells versus control. Echocardiography may be used to assess improvement in ventricular function. Baseline echocardiography may be performed 72 h following coronary artery ligation; subsequent testing may be performed 4 weeks later
30 to evaluate ventricular function. Necropsy examination upon sacrifice of the

- 59 -

test animal allows for histological evaluation of capillary density, as well as extent of infarction and scar formation. Revascularization and repair of ischemic damage in limbs is assessed, for example, by comparing capillary density and blood flow in animals treated with TC-PTP deficient cells versus
5 control. Animals administered TC-PTP deficient cells have increased capillary density and better blood flow than control animals injected with PBS and TC-PTP^{+/+} bone marrow cells. This may be evaluated using Doppler ultrasonography, performed immediately after surgery and repeated every 3 to 5 d up to 5 weeks post surgery. Alternatively, angiography, consisting of
10 intravenous injection of radiopaque contrast agent followed by X-ray imaging, may be used to visualize neovascularization. Necropsy examination supplements the above imaging modalities upon sacrifice of the test animal.

Ex- vivo cultured EPC and CEPC

- 15 **[00173]** Operated mice are injected with cultured wild type Balb/c bone marrow, treated 48h with TC-PTP-specific RNAi sequence to increase the number of bone marrow EPC and CEPC, one day post-surgery. Cells are injected i.v. (tail vein; 1 x 10E6 cells in PBS). The control mice are injected with PBS or Scrambled (SCR) RNAi sequence treated bone marrow cells.
- 20 **[00174]** Other operated mice are injected with cultured wild type Balb/c bone marrow, treated 48h with small molecule inhibitor of TC-PTP (10µM and 50µM concentration) to increase the number of bone marrow EPC and CEPC, one day post-surgery. Cells are injected i.v. (tail vein; 1 x 10E6 cells in PBS). The control mice are injected with DMSO.
- 25 **[00175]** Revascularization and repair of ischemic heart is assessed by comparing the degree of recovery from the induced ischemic damage in animals treated with cells inhibited for TC-PTP versus control. Echocardiography may be used to assess improvement in ventricular function. Baseline echocardiography may be performed 72 h following
30 coronary artery ligation; subsequent testing may be performed 4 weeks later

- 60 -

to evaluate ventricular function. Necropsy examination upon sacrifice of the test animal allows for histological evaluation of capillary density, as well as extent of infarction and scar formation. Revascularization and repair of ischemic damage is assessed, for example, by comparing capillary density and blood flow in animals treated with cells inhibited for TC-PTP versus control. Animals administered cells inhibited for TC-PTP have increased capillary density and better blood flow than control animals injected with PBS and TC-PTP^{+/+} bone marrow cells. This may be evaluated using Doppler ultrasonography, performed immediately after surgery and repeated every 3 to 5 d up to 5 weeks post surgery. Alternatively, angiography, consisting of intravenous injection of radiopaque contrast agent followed by X-ray imaging, may be used to visualize neovascularization. Necropsy examination supplements the above imaging modalities upon sacrifice of the test animal.

15 Local Administration

[00176] Operated mice, after the ligation (either femoral artery or LAD) and before closing the muscles and skin, are administered TC-PTP^{-/-} bone marrow cells, which are injected directly at the ligation site (1 x 10E6 cells in PBS). The control mice undergo the same procedure with PBS or TC-PTP^{+/+} bone marrow cells injected at the ligation site instead of cell suspension.

[00177] Operated mice, after the ligation (either femoral artery or LAD) and before closing the muscles and skin, are administered cultured wild type Balb/c bone marrow, treated 48h with TC-PTP-specific RNAi sequence to increase the number of bone marrow EPC and CEPC, directly at the ligation site (1 x 10E6 cells in PBS). The control mice undergo the same procedure with PBS, or Balb/c bone marrow treated with SCR RNAi sequence injected at the ligation site instead of RNAi-treated cell suspension.

[00178] Operated mice, after the ligation (either femoral artery or LAD) and before closing the muscles and skin, are administered cultured wild type Balb/c bone marrow, treated 48h with small molecule inhibitor of TC-PTP

- 61 -

(10 μ M and 50 μ M concentration) to increase the number of bone marrow EPC and CEPC are injected directly at the ligation site (1 x 10E6 cells in PBS). The control mice undergo the same procedure with DMSO injected at the ligation site instead of a treated cell suspension.

- 5 **[00179]** Revascularization and repair of ischemic heart is assessed by comparing the degree of recovery from the induced ischemic damage in animals treated with TC-PTP inhibited cells versus control. Echocardiography may be used to assess improvement in ventricular function. Baseline echocardiography may be performed 72 h following coronary artery ligation;
- 10 subsequent testing may be performed 4 weeks later to evaluate ventricular function. Necropsy examination upon sacrifice of the test animal allows for histological evaluation of capillary density, as well as extent of infarction and scar formation. Revascularization and repair of ischemic damage is assessed, for example, by comparing capillary density and blood flow in animals treated
- 15 with TC-PTP inhibited cells versus control. Animals administered TC-PTP inhibited cells have increased capillary density and better blood flow than control animals injected with TC-PTP^{+/+} bone marrow cells. This may be evaluated using Doppler ultrasonography, performed immediately after surgery and repeated every 3 to 5 d up to 5 weeks post surgery. Alternatively,
- 20 angiography, consisting of intravenous injection of radiopaque contrast agent followed by X-ray imaging, may be used to visualize neovascularization. Necropsy examination supplements the above imaging modalities upon sacrifice of the test animal.

25 **Systemic Administration**

[00180] Immediately post-surgery, TC-PTP blocking agent (either RNAi sequence or small molecule inhibitor) is administered i.v. via tail vein injection. Control animals are administered an appropriate control agent (e.g. PBS and/or DMSO).

- 62 -

Revascularization and repair of ischemic heart is assessed by comparing the degree of recovery from the induced ischemic damage in animals treated with TC-PTP blocking agent or inhibitor versus control. Echocardiography may be used to assess improvement in ventricular function. Baseline
5 echocardiography may be performed 72 h following coronary artery ligation; subsequent testing may be performed 4 weeks later to evaluate ventricular function. Necropsy examination upon sacrifice of the test animal allows for histological evaluation of capillary density, as well as extent of infarction and scar formation. Revascularization and repair of ischemic damage is assessed,
10 for example, by comparing capillary density and blood flow in animals treated with TC-PTP blocking agent versus control. Animals with coronary artery ligation administered TC-PTP blocking agent will have increased capillary density and better blood flow than control animals injected with PBS or DMSO. These animals will also have increased left ventricular function.
15 Animals with femoral artery ligation administered TC-PTP blocking agent will regain use of their leg. This may be evaluated using Doppler ultrasonography, performed immediately after surgery and repeated every 3 to 5 d up to 5 weeks post surgery. Alternatively, angiography, consisting of intravenous injection of radiopaque contrast agent followed by X-ray imaging, may be
20 used to visualize neovascularization. Necropsy examination supplements the above imaging modalities upon sacrifice of the test animal.

Example 8

[00181] Increasing the pool of stem cells accelerates the regeneration of
25 the individual's own immune system. Animal model of leukemias (chronic myeloid leukemia (CML) BCR-Abl transformed cells), lymphomas (B-cells non-hodgkin p53-induced) and myeloma will be used to test for increases in bone marrow stem cell content to accelerate regeneration of a healthy immune system. In all the animal models of hematopoietic cancers, remission
30 is achieved by transplantation of normal healthy bone marrow post-chemotherapy and radiation therapy. Immediately post-bone marrow

- 63 -

transplant, all animals in experimental group #1 are submitted to an intensive daily gavage regimen of the small molecule inhibitor or control DMSO. Experimental group #2 are receiving daily i.v. injection (tail vein) of the small molecule inhibitor or DMSO control. Both treatment regimens are
5 administered for 1 month. Each mouse is followed post-transplant by taking saphenous vein blood and performing complete blood count and flow cytometry analysis looking for the extend of leukocytosis as a sign of hematopoietic malignancy and the development of the new immune donor system. Animals that received the small molecule inhibitor should have
10 improved healing time and achieve faster remission.

[00182] Similar regimen can also be employed in individuals with immunodeficiencies such as congenital neutropenia and severe combined immunodeficiency syndrome or lack/abnormal red blood cell production (anemia and thalassemia).

15 **[00183]** Tissue resident stem cells exist and are thought to play an important role in regeneration. For example, cardiac resident stem cells can be isolated with cell surface markers by flow cytometry and contribute to the recovery post-ischemia. In animal model of myocardial infarct after the ligation of the LAD and before closing the muscles and skin, small molecule inhibitor
20 are injected directly at the ligation site to augment the pool of resident cardiac stem cells. The control mice will undergo the same procedure, but DMSO is injected at the ligation site instead of the compound.

Example 9

25 **[00184]** Together, Hematopoietic stem cells (HSC) and endothelial progenitor cells (EPC) represent 0.05% of total bone marrow cellularity and are, at the present time, indistinguishable from each other. As depicted in Figure 1, these rare cells can be subdivided based on differential expression of cell surface markers.

- 64 -

[00185] The function of T cell protein tyrosine phosphatase (TC-PTP) in bone marrow hematopoietic and endothelial stem cells was studied by flow cytometry analysis of bone marrow from TC-PTP^{-/-} mice. These stem cells are defined by surface expression of CD117 and Sca-1, and by lack of expression of an arbitrary set of surface markers found on terminally differentiated cells, together termed lineage (Lin) markers and multipotent progenitor cell markers. Lin markers included CD3, CD4, CD5, CD8, CD11b, CD19, CD31, CD49b, and Ter119, and multipotent progenitor markers are CD127 and CD135. This pluripotent stem cell population was further fractionated based on surface expression of CD105. The total number of stem cells was increased 5-fold, and the number of CD105⁺ stem cells was increased 9-fold in TC-PTP^{-/-} bone marrow compared to control. In addition, a new population of stem cells expressing high levels of Sca-1 was observed.

An *in vitro* differentiation assay for endothelial cells (EC) was performed with purified CD105⁻ and CD105⁺ stem cells to quantify the number of endothelial progenitor cells (EPC) in these populations. The number of EPC was increased 5-fold in the bone marrow of TC-PTP^{-/-} mice compared to control, and was increased 7-fold when purified CD105⁺ stem cells were used, whereas few EC colonies were obtained from CD105⁻ stem cells. Further, flow cytometry analysis of whole bone marrow showed a 50% increase in the number of circulating EPC in TC-PTP^{-/-} mice compared to control. To determine whether the increase in stem cell numbers was attributable to lack of TC-PTP enzymatic activity, bone marrow cultures were incubated in the presence of orthovanadate, a nonspecific phosphatase inhibitor. The number of stem cells was increased 1.8-fold after 24 h, and 9-fold after 48h of culture with 50 μ M orthovanadate. Together, these results demonstrate a role for TC-PTP in regulating both hematopoietic and endothelial stem cell proliferation, and suggest the therapeutic use of agents that inhibit TC-PTP to augment stem cell populations.

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Example 10

Nucleic Acid Inhibition

Materials and Methods

[00186] Applicable materials and methods are used as described in Example 1.

- 5 **[00187]** Nucleic acid molecules suitable to inhibit TC-PTP are first chosen and tested. Suitable nucleic acid molecules are chosen using rules and/or computer programs known in the art. Electroporation of antisense or RNAi nucleic acids is accomplished using methods known in the art such as those described in Genesis 2003 Aug: 36(4):203-8. Decreased expression of
- 10 TC-PTP is confirmed using methods known in the art such as northern blot and RT-PCR.

[00188] While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be

15 understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

[00189] All publications, patents and patent applications are herein

20 incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

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- 71 -

CLAIMS:

1. A method for expanding stem cells comprising administering an effective amount of an agent that inhibits TC-PTP to a starting population of cells comprising stem cells, whereby inhibition of TC-PTP increases stem cell expansion in a final population of cells.
5
2. The method of claim 1 where the agent that inhibits TC-PTP is selected from the group consisting of phosphatase inhibitors, small molecule inhibitors, nucleic acid molecules and antibodies.
3. The method of claim 2 wherein the phosphatase inhibitor is a vanadate
10 compound.
4. The method of claim 3 wherein the vanadate compound is selected from the group consisting of vanadate, orthovanadate, pervanadate; vanadate dimer, vanadate tetramer, vanadate pentamer, vanadate hexamer, vanadate heptamer, vanadate octamer, vanadate nonamer, vanadate decamer,
15 vanadate polymer, vanadyl sulfate, bis (6, ethylpicolinate) (H₂O)₂ oxovanadium(IV) complex, bis (1-oxy-2- pyridinethiolato) oxovanadium (IV), bis (maltolato) oxovanadium (IV), bis (biguanidato) oxovanadium (IV), bis (N,N'-dimethylbiguanidato) oxovanadium (IV), bis (beta-phenethyl-biguanidato) oxovanadium (IV), and any salts thereof.
- 20 5. The method of claim 3 wherein the vanadate compound is orthovanadate or a salt thereof.
6. The method of claim 2 wherein the nucleic acid molecule is an antisense nucleic acid molecule.
7. The method of claim 2 wherein the nucleic acid is a double stranded
25 nucleic acid molecule.
8. The method of claim 2 wherein the nucleic acid is a TC-PTP specific RNAi sequence.

- 72 -

9. The method of claim 8 wherein the TC-PTP specific RNAi sequence is GCCCAUAUGAUCACAGUCG (SEQ ID NO:1).
10. The method of claim 8 wherein the TC-PTP specific RNAi sequence is GGCACAAAGAAGUUACAUC (SEQ ID NO:2).
- 5 11. The method of claim 8 wherein the TC-PTP specific RNAi sequence is GCCCAUAUGAUCACAGUCGtt (SEQ ID NO:3).
12. The method of claim 8 wherein the TC-PTP specific RNAi sequence is GGCACAAAGAAGUUACAUCtt (SEQ ID NO:4).
13. The method of claim 2 wherein the small molecule inhibitor is a non-
10 selective phosphatase.
14. The method of claim 2 wherein the small molecule inhibitor is a thioxothiazolidinone compound of Formula I or a pharmaceutically acceptable salt thereof.
15. The method of claim 14 wherein the thioxothiazolidinone is a bis-aryl
15 thioxothiazolidinone.
16. The method of claim 15 wherein the bis-aryl thioxothiazolidinone is 5-[(4-hydroxy-3-methoxy-5-nitrophenyl)methylene]-3-(*m*-chlorophenyl)-2-thioxo-4-thiazolidinone or a pharmaceutically acceptable salt thereof.
17. The method of any one of claims 1-16 wherein the starting population
20 of cells is selected from blood, bone marrow, umbilical cord, lymphoid tissue, epithelia, thymus, liver, spleen, cancerous tissues, lymph node tissue, infected tissue, fetal tissue and fractions or enriched portions thereof.
18. The method of claim 17 wherein the starting population of cells is bone marrow.
- 25 19. The method of claim 17 or 18 wherein the starting population of cells is human.

- 73 -

20. The method of any one of claims 17-19 wherein the starting population of cells comprises adult stem cells.
21. The method of any one of claims 17-19 wherein the starting population of cells comprises fetal stem cells.
- 5 22. The method of any one of claims 17-21 wherein the starting population of cells is enriched for or depleted of certain cell types prior to incubating the starting population with an agent that inhibits TC-PTP.
23. The method of claim 22 wherein the starting population is depleted of cell types expressing terminal differentiation markers.
- 10 24. The method of claim 23 wherein the terminal differentiation markers and multipotent progenitor markers are selected from the group CD3, CD4, CD5, CD8, CD11b, CD19, CD49b, Ter119, CD31, CD127 and CD135.
25. The method of claim 23 wherein the terminal differentiation markers and multipotent progenitor markers are selected from the group comprising
15 CD235a, CD3, CD4, CD5, CD8, CD11b, CD19, CD49b, CD31, CD127 and CD135.
26. The method of claim 22 or 23 wherein the starting population is enriched for cell types expressing cell markers present on stem cells.
27. The method of claim 26 wherein the cell markers present on stem cells
20 are chosen from the group CD117, Sca-1, CD14, CD105 and combinations thereof.
28. The method of claim 26 wherein the cell markers present on stem cells are chosen from the group CD117, CD133, CD34, CD14, CD105, and combinations thereof.
- 25 29. The method of any one of claims 1-28 wherein the agent that inhibits TC-PTP is administered ex vivo.

- 74 -

30. The method of any one of claims 1-28 wherein the agent that inhibits TC-PTP is administered in vivo.
31. The method of any one of claims 1-28 wherein the starting population of cells is peripheral blood.
- 5 32. The method of claim 29 wherein the starting population of cells is a cell culture.
33. A method of claims 1-32 further comprising the step of:
- a) isolating a population of expanded stem cells from the final population of cells.
- 10 34. The method of claim 33 wherein the population of expanded stem cells is isolated using a substance that binds one or more cell markers selected from the group consisting of CD117, Sca-1 and CD105.
35. The method of claim 34 wherein the population of expanded stem cells is isolated using substances that bind cell markers CD117 and Sca-1.
- 15 36. The method of claim 35 wherein the population of expanded stem cells is isolated using substances that bind cell markers CD117, Sca-1 and CD105.
37. The method of any one of claims 34-36 where Sca-1 expression is high.
38. The method of claim 33 wherein the population of expanded stem cells
20 is isolated using a substance that binds one or more cell markers selected from the group consisting of CD117, CD34, CD133, CD14 and CD105.
39. The method of claim 38 wherein the population of expanded stem cells is isolated using substances that bind CD117, CD34, CD133 and CD105 cell markers.

- 75 -

40. The method of claim 38 wherein the population of expanded stem cells is isolated using substances that bind CD117, CD34, CD133, CD14 and CD105 cell markers.

41. The method of any one of claims 1 to 40 wherein the population of
5 expanded cells is EPC.

42. The method of any one of claims 33-41 wherein the population of expanded cells is EPC and wherein the EPC are isolated using substances that bind CD117, CD34, CD133 cell markers.

43. The method of claim 44 further comprising using a substance that
10 binds a CD105 cell marker to isolate the EPC.

44. The method claim 42 wherein the EPC are CEPC.

45. The method of claim 44 wherein the CEPC are isolated using substances that bind CD117, CD34, CD133 and CD14 cell markers.

46. The method of claim 45 further using a substance that binds a CD105
15 cell marker.

47. The method of claim 33 wherein the step of isolating a population of expanded stem cells comprises:

- i. fractionating the final population of cells to obtain a sub-population of cells negative for one or more terminal differentiation cell markers and/or multipotent progenitor markers; and
 - ii. fractionating said sub-population of cells to obtain a stem cell enriched fraction using a substance that binds one or more cell markers selected from the group CD117, Sca-1, CD34, CD133 and CD105.
- 20
25

48. The method of claim 47 wherein the terminal differentiation markers and multipotent progenitor markers are selected from the group CD3, CD4,

- 76 -

CD5, CD8, CD11b, CD19, CD49b, Ter119, CD235a CD31, CD127 and CD135.

49. The method of claim 47 or 48 wherein the sub-population is fractionated using substances that bind cell markers comprising CD117 and
5 Sca-1.

50. The method of any one of claims 47-49 wherein Sca-1 expression is high.

51. The method of any one of claims 47-50 wherein the sub-population is fractionated using substances that bind cell markers comprising CD117, Sca-
10 1 and CD105 and wherein the stem cell enriched fraction is enriched with endothelial precursor cells.

52. The method of claim 51 wherein the sub-population is further fractionated using a substance that binds CD14 cell marker.

53. The method of claim 47 or 48 wherein the sub-population is
15 fractionated using substances that bind cell markers comprising CD117, CD133 and CD34.

54. The method of any one of claim 47, 48 or 53 wherein the sub-population is fractionated using substances that bind cells markers comprising CD117, CD133, CD34 and CD105 and wherein the stem cell enriched fraction
20 is enriched with endothelial precursor cells.

55. The method of claim 54 wherein the sub-population is further fractionated using a substance that binds cell marker CD14.

56. Use of a substance that binds to CD105 to isolate endothelial progenitor cells.

25 57. The use of claim 56 further comprising using substances that bind CD117 and Sca-1 cell markers.

- 77 -

58. The use of claim 56 further comprising using substances that bind CD117, CD34 and CD133 cell markers.

59. The use of claim 58 further comprising using a substance that binds CD14 cell marker.

5 60. The use of any one of claims 56-58 further comprising fractionating the population of cells comprising stem cells using terminal differentiation cell markers and/or multipotent progenitor markers, wherein the terminal differentiation markers and multipotent progenitor markers are selected from CD3, CD4, CD5, CD8, CD11b, CD19, CD49b, Ter119, CD235a, CD31,
10 CD127 and CD135.

61. The use of any one of claims 56-60 wherein the EPC are isolated from a population of cells wherein the population of cells is selected from blood, bone marrow, umbilical cord, lymphoid tissue, epithelia, thymus, liver, spleen, cancerous tissues, lymph node tissue, infected tissue, fetal tissue and
15 fractions or enriched portions thereof.

62. An isolated EPC cell that has been isolated according to any one of claims 1-55 that expresses CD105.

63. A method of establishing a stem cell line comprising incubating a starting population of cells comprising stem cells with an effective amount of
20 an agent that inhibits TC-PTP.

64. A culture medium for ex vivo expansion of stem cells comprising an agent that inhibits TC-PTP.

65. The culture medium of claim 64 wherein the agent that inhibits TC-PTP is selected from a phosphatase inhibitor, a vanadate compound, a small
25 molecule, an antisense nucleic acid, and a RNAi sequence.

- 78 -

66. Use of isolated endothelial precursor cells for revascularizing ischemic tissue wherein the endothelial precursor cells are isolated using a method of any one of claims 33-55.
67. Use of isolated endothelial precursor cells for treating occluded
5 coronary arteries wherein the endothelial precursor cells are isolated using a method of any one of claims 33-55.
68. Use of an agent that inhibits TC-PTP for increasing stem cells in an animal in need thereof.
69. Use of an agent that inhibits TC-PTP for the manufacture of a
10 medicament for increasing stem cells in an animal in need thereof.
70. The use of any one of claims 68 or 69 for enhancing revascularization comprising administering an agent that inhibits TC-PTP to an animal in need thereof.
71. The use of claim 68 or 69 for the manufacture of a medicament for
15 enhancing revascularization.
72. The use of claim 68 or 69 for treating ischemia.
73. The use of claim 72 wherein the ischemia is acute.
74. The use of claim 68 or 69 for treating a vessel disease.
75. The use of claim 68 or 69 for treating a hematopoietic cell disorder.
- 20 76. The use of claim 68 or 69 wherein the animal receives a bone marrow transplant.
77. The use of claim 68 or 69 wherein the agent that inhibits TC-PTP is a small molecule inhibitor.
78. The use of claim 68 or 69 wherein the small molecule inhibitor is a
25 thioxothiazolidinone compound.

- 79 -

79. The use of claim 68 or 69 wherein the agent is 5-[(4-hydroxy-3-methoxy-5-nitrophenyl)methylene]-3-(*m*-chlorophenyl)-2-thioxo-4-thiazolidinone.

80. The use of claim 68 or 69 wherein the agent that inhibits TC-PTP is a
5 RNAi sequence.

81. The use of claim 68 or 69 wherein the agent that is a vanadate compound.

82. The use of claim 68 or 69 wherein the agent is administered locally.

83. The use of claim 68 or 69 wherein the agent is administered
10 systemically.

84. A method of identifying substances which can inhibit TC-PTP comprising the steps of:

- 15
- (a) reacting TC-PTP and a test substance, under conditions that permit the test substance to interact with TC-PTP; and
 - (b) assaying for TC-PTP activity, wherein decreased TC-PTP activity relative to control indicates the test substance is capable of inhibiting TC-PTP.

1/7

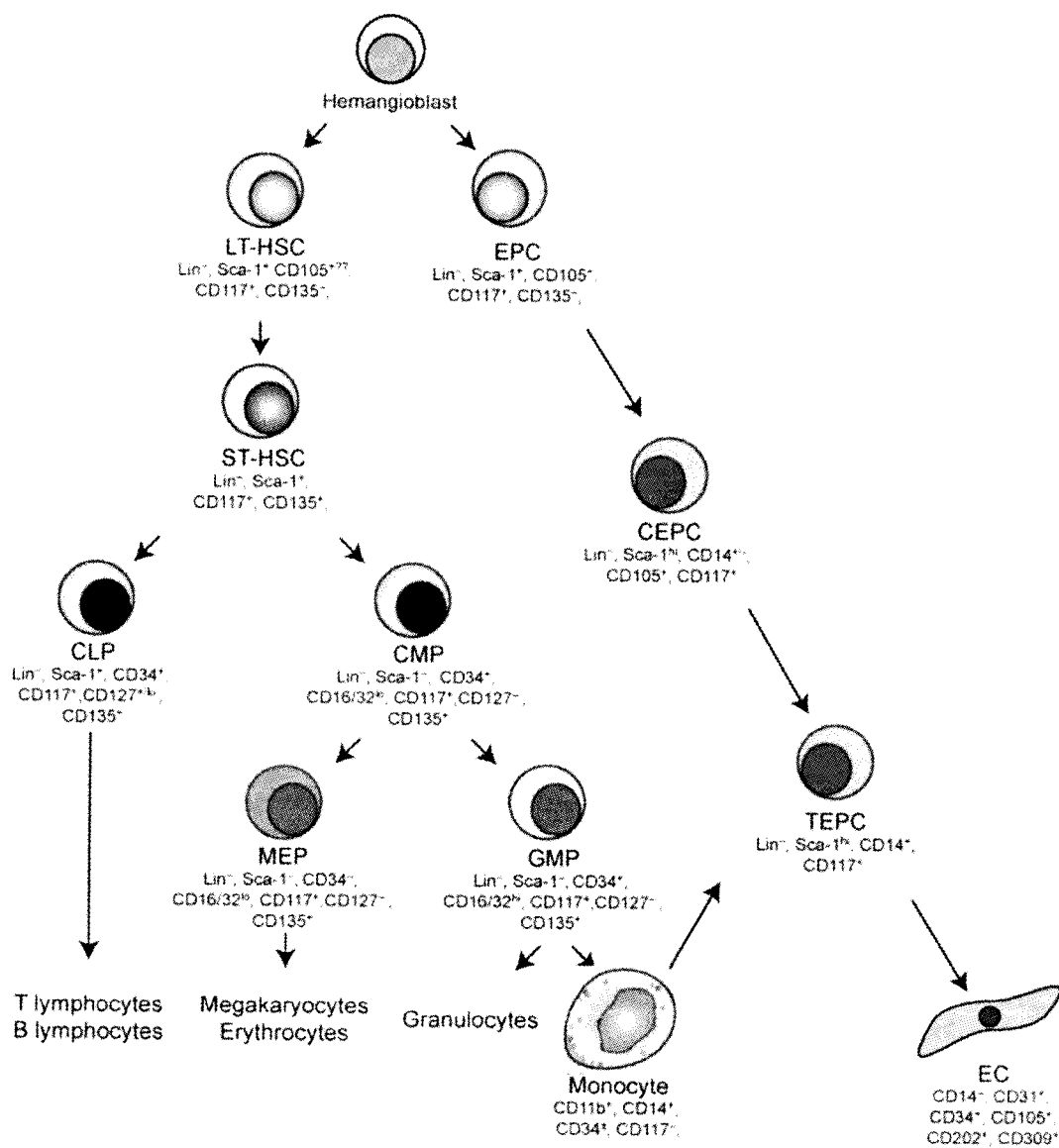


FIGURE 1

2/7

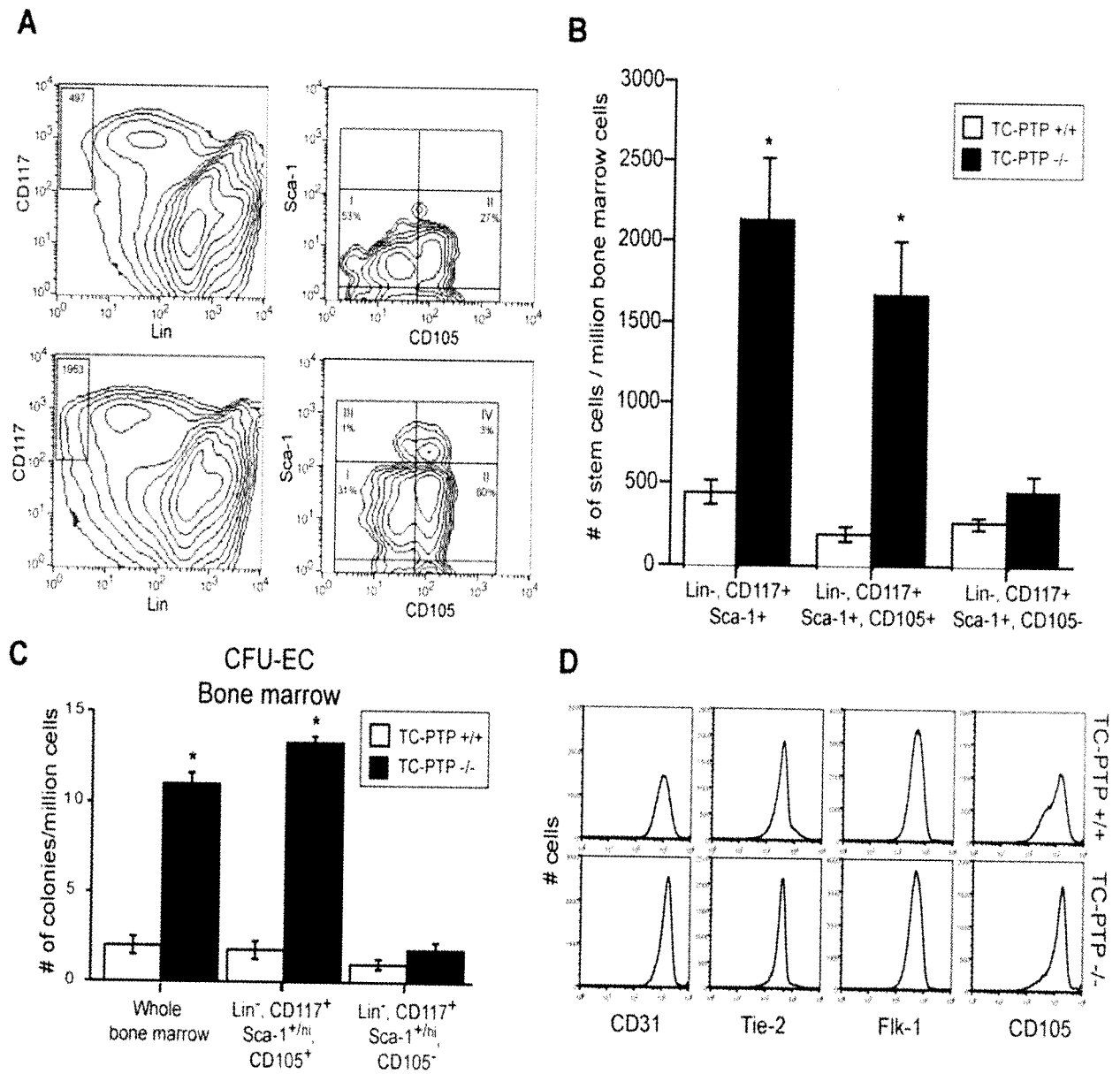


FIGURE 2

3/7

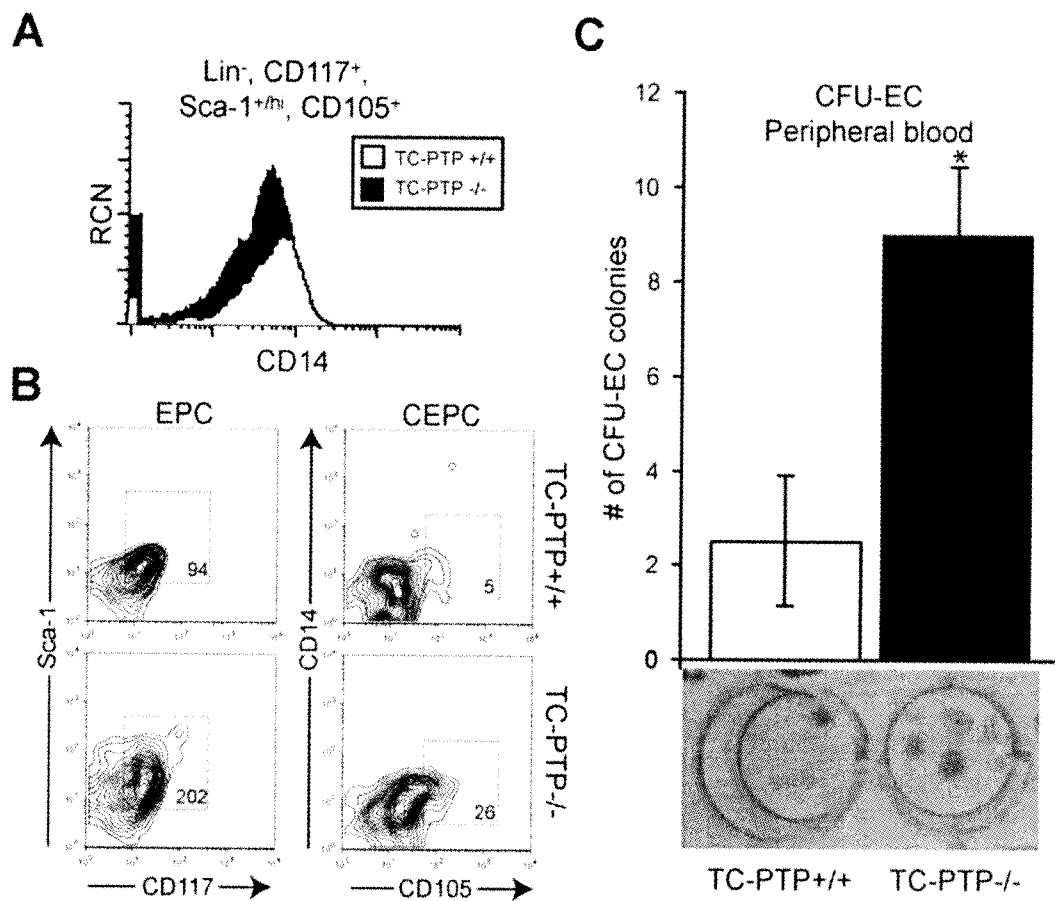


FIGURE 3

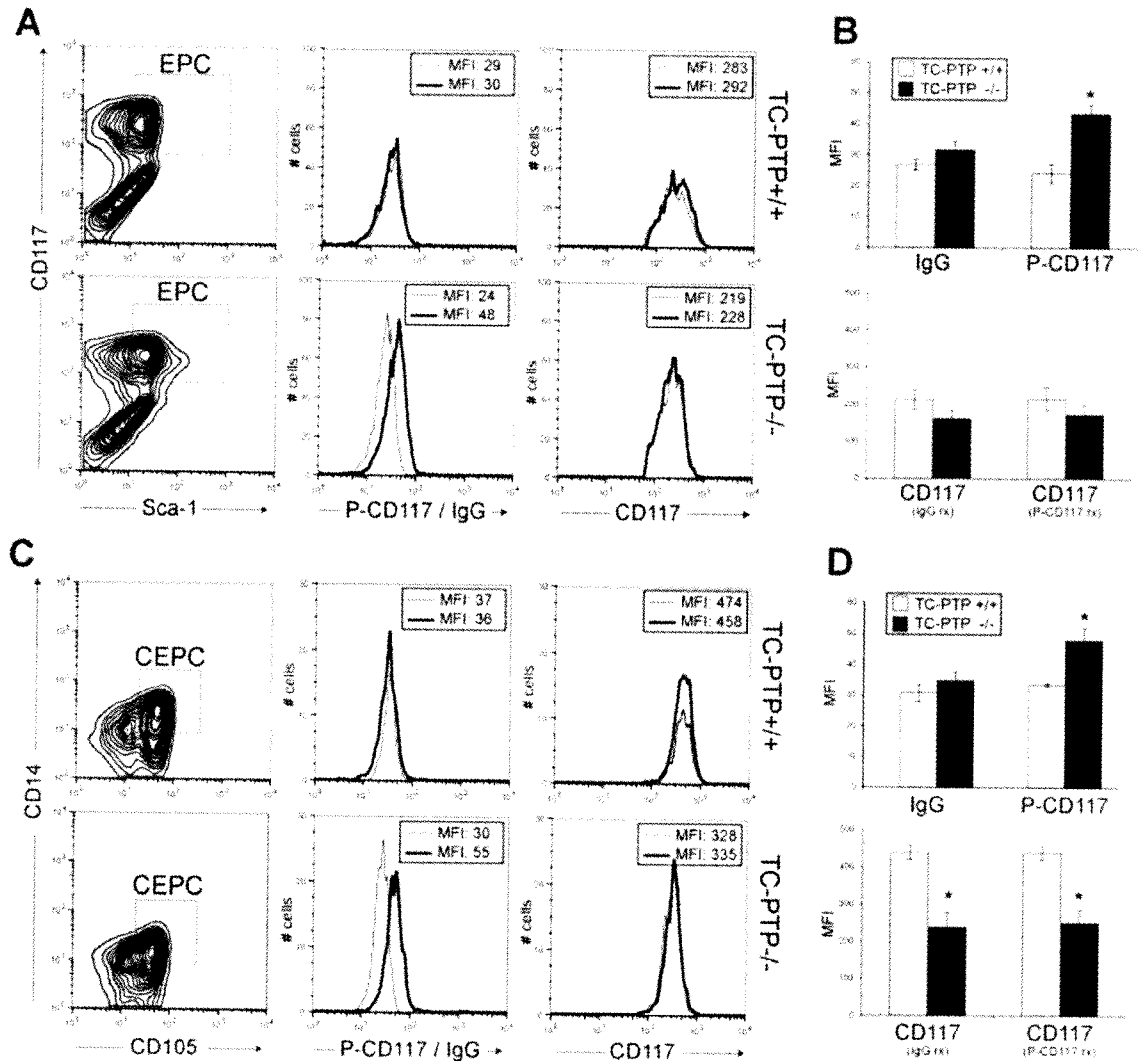


FIGURE 4

5/7

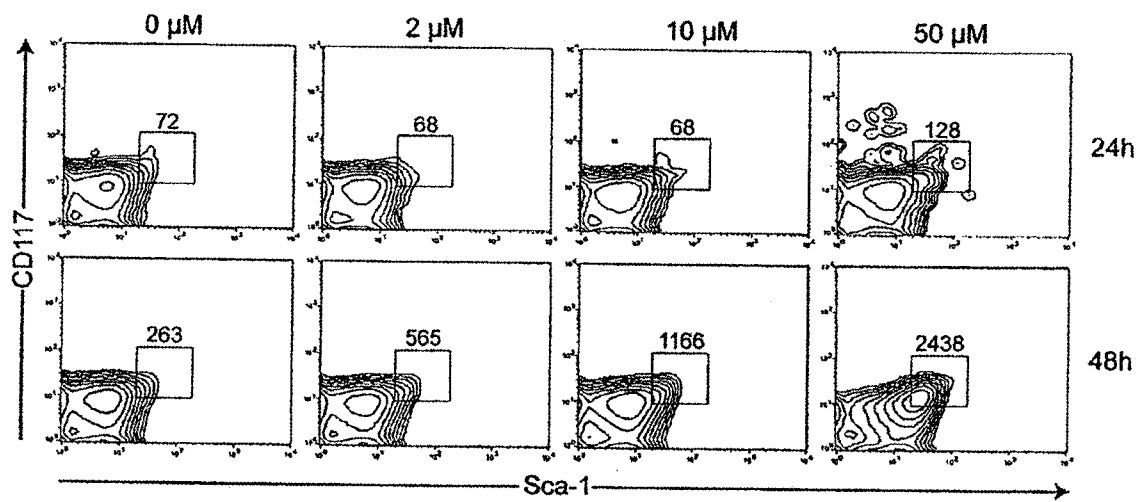


FIGURE 5

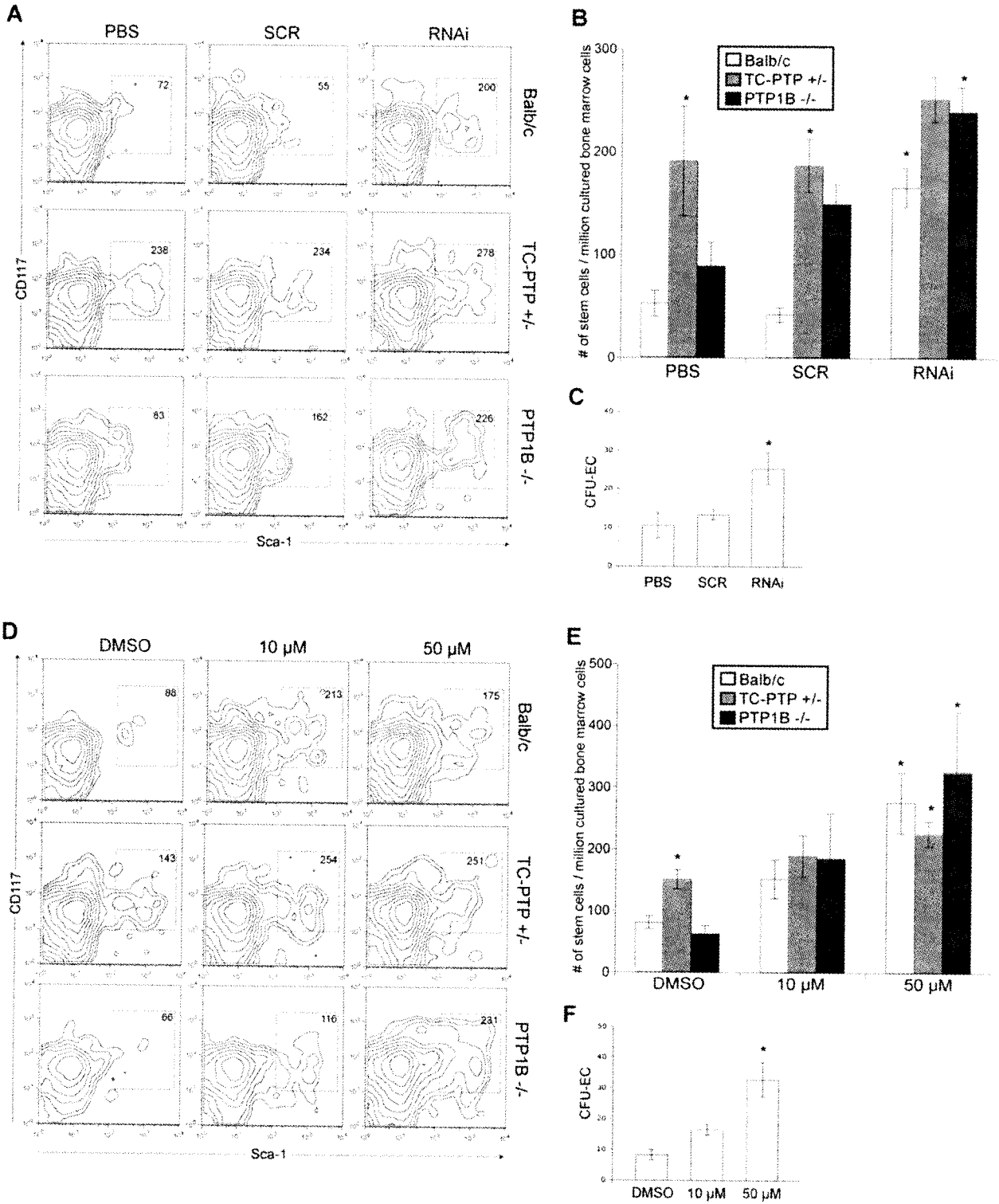


FIGURE 6

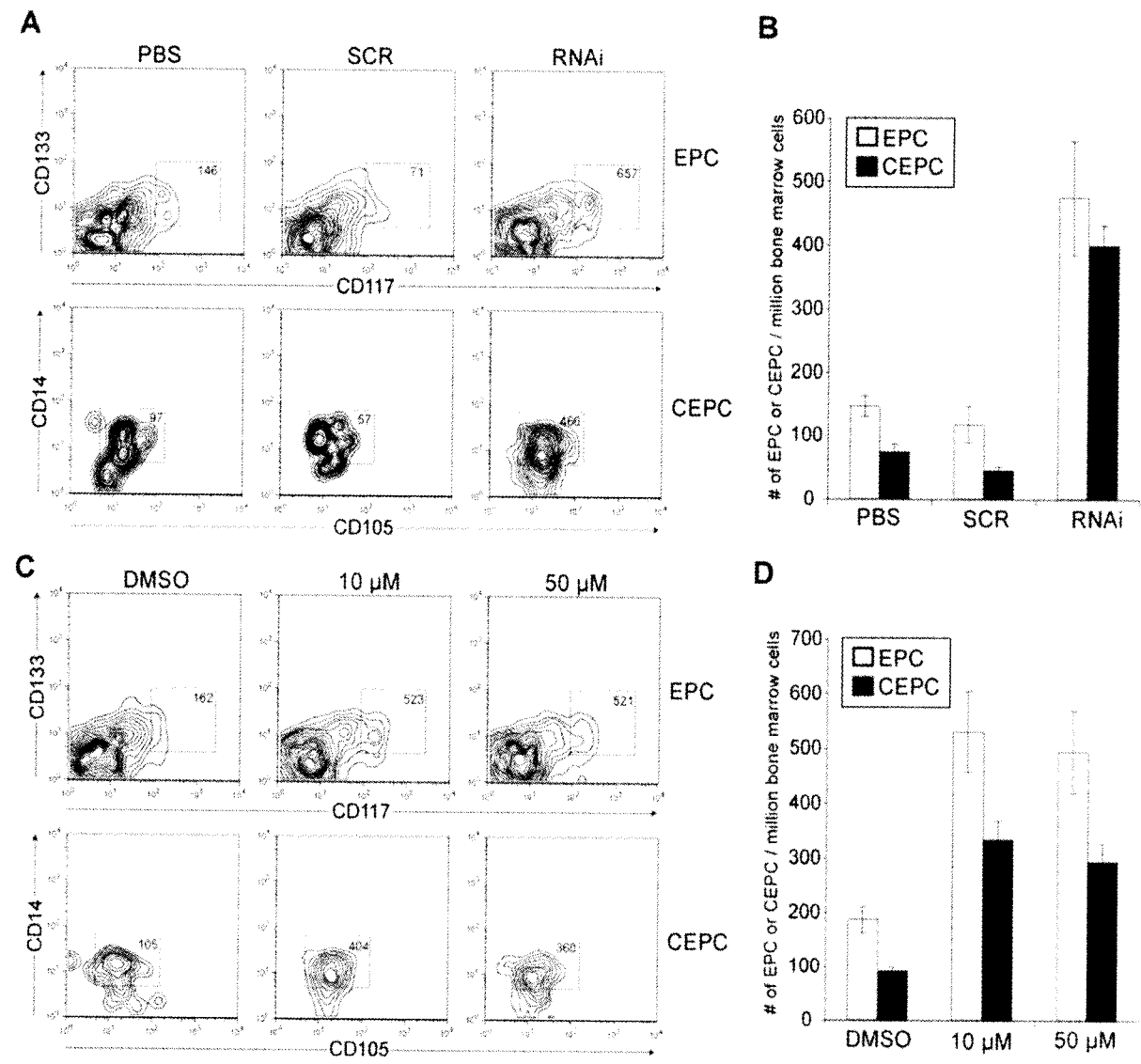


FIGURE 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2007/001819

A. CLASSIFICATION OF SUBJECT MATTER

IPC: *C12N 5/06* (2006.01) , *A61K 31/426* (2006.01) , *A61K 31/7105* (2006.01) , *A61K 33/24* (2006.01) , *A61K 35/44* (2006.01) , *A61K 48/00* (2006.01) (more IPCs on the last page)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: *C12N 5/06* (2006.01) , *A61K 31/426* (2006.01) , *A61K 31/7105* (2006.01) , *A61K 33/24* (2006.01) , *A61K 35/44* (2006.01) , *A61K 48/00* (2006.01) (more IPCs on the last page)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

databases: Delphion, PubMed, SCOPUS

search terms: T cell protein tyrosine phosphatase (TC-PTP), stem cells, endothelial progenitor cells, endoglin, CD105

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/018655 A2 (NEURONOVA AB) 4 March 2004 see whole document	1 to 5, 17, 19 to 21, 29, 32, 63 to 65
A	SIMONCIC PD et al. PTP1B and TC-PTP: novel roles in immune-cell signaling. CAN J PHYSIOL PHARMACOL. July 2006, Vol. 84, No. 7, pages 667-675	6 to 16, 18, 22 to 28, 30, 31, 33 to 55, and 68 to 83
A	YOU-TEN KE et al. Impaired Bone Marrow Microenvironment and Immune Function in T Cell Protein Tyrosine Phosphatase-deficient Mice. J EXP MED. August 1997, Vol. 186, No. 5, pages 683-693	6 to 16, 18, 22 to 28, 30, 31, 33 to 55, and 68 to 83
X	URBICH C and DIMMELER S. Endothelial Progenitor Cells: Characterization and Role in Vascular Biology. CIRC RES. August 2004, Vol. 95, No. 4, pages 343-353	62, 66 and 67
A	see whole document	56 to 61

[X] Further documents are listed in the continuation of Box

[X] See patent family annex.

* Special categories of cited documents :	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

7 January 2008 (07-01-2008)

Date of mailing of the international search report

23 January 2008 (23-01-2008)

Name and mailing address of the ISA/CA
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Authorized officer

Cynthia Bruce-Payne 819-997-4921

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2007/001819

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant	Relevant to claim No.
A	CHEN CZ et al. Identification of endoglin as a functional marker that defines long-term repopulating hematopoietic stem cells. PNAS. November 2002, Vol. 99, No. 24, pages 15468-15473	56 to 61

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/CA2007/001819**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. ☒ Claim Nos. : 1 to 28, 30 to 55, 70, 76, 82 and 83
because they relate to subject matter not required to be searched by this Authority, namely :

Although claims 1 to 28, 30 to 55, 70, 76, 82 and 83 encompass a method of treatment of the human/animal body which this Authority is not obliged to search under Rule 39.1(iv) of the PCT, the search has been carried out based on the alleged effects of an agent that inhibits TC-PTP referred to therein.
2. ☐ Claim Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :
3. ☐ Claim Nos. :
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

Group A - Claims 1 to 55, 62(in part), and 63 to 83 featuring the expansion of stem cells following administration of an agent that inhibits T-cell protein tyrosine phosphatase (TC-PTP) as well as the use of isolated and expanded cells.

Group B - Claims 56 to 61 and 62(in part) featuring the use of a substance that binds to CD105 to isolate endothelial progenitor cells.

Group C - Claim 84 featuring a method of identifying substances which can inhibit TC-PTP.

continued: see extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. : 1 to 83
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☒ No protest accompanied the payment of additional search fees.

Continuation of: page 3, Box No. III

A special technical feature common to all claims is lacking. For instance, Group A pertaining to the use of an agent which inhibits TC-PTP is not so linked as a single general inventive concept to the use of a substance that binds to CD105 in Group B. Likewise, Group C is neither linked to Group A nor Group B inventions.

Continuation of: page 4, Boxes A and B

A61P 9/00 (2006.01) , *A61P 9/10* (2006.01) , *C12N 5/08* (2006.01) , *G01N 33/53* (2006.01) ,
C12N 9/16 (2006.01) , *C12N 9/99* (2006.01)

INTERNATIONAL SEARCH REPORT
Information on patent family members

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Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
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