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(54) **Title:** METHODS FOR INCREASING THE NUMBER OF CIRCULATING CELLS

(57) **Abstract:** The present invention relates to a method for mobilisation of cells capable of circulating around the body of a subject, said method comprising administering to the subject an effective amount of low molecular weight anionic glycan mimetic, or a pharmaceutically acceptable salt thereof.

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METHODS FOR INCREASING THE NUMBER OF CIRCULATING CELLS

Technical Field

The present invention relates to methods for increasing the number of circulating cells. More particularly, the invention relates to methods for increasing the number of circulating stem cells and leukocytes using anionic glycan mimetics.

Background

The migration and homing of endogenous pluripotent stem cells from the bone marrow has been shown to play an important role in the repair and regeneration of various tissues and organs throughout the human body. In addition, the controversy existing over the use of embryonic stem cells in tissue regeneration has further increased the interest in developing treatments utilising adult stem cells. In many experiments using rodents, it has been shown that adult stem cells can participate in tissue and organ regeneration in almost all lesions.

There has been considerable focus on the ability of bone-marrow derived haematopoietic stem cells (HSCs, immature cells of the immune system) to differentiate into non-haematopoietic cells of various lineages. Peripheral blood stem cells (PBSC) have become the preferred source of stem cells for autologous transplantation because of the technical advantage and the shorter time to engraftment. The mobilisation of HSCs is now routinely used in the clinic as a source of haematopoietic stem cells for bone marrow transplantation, and the potential exists for stem cell therapy for tissue regeneration. Mobilisation into the peripheral blood is currently achieved with chemotherapy (eg cyclophosphamide), or by administration of expensive haematopoietic growth factors such as granulocyte colony-stimulating factor (G-CSF) or granulocyte macrophage colony-stimulating factor (GM-CSF), which requires days to achieve peak circulating HSC numbers, as well as short-lived IL-8 therapy which can act in minutes, but its effect is short lived. However, up to 25% of patients undergoing autotransplantation for haematologic malignancies have poor stem cell mobilisation, responding poorly to initial G-CSF mobilisation, and requiring subsequent higher dose cytokines or combination therapy (with increased cost and risk of infection).

Recently, the present inventors have shown that mobilisation of mature leukocytes, particularly eosinophils, can provide a beneficial response in fighting cancer.

Mature leukocytes and PBCs can be mobilised from the bone marrow into the circulation by the addition of sulfated polysaccharides, such as heparin and fucoidin and the sulfated oligosaccharide dextran sulfate. However, each of these compounds can also act to prevent entry of the cells into tissues through inhibition of initial selectin-adhesion molecule binding to the endothelium and activity of extracellular matrix (ECM)-degrading enzymes preventing migration through the endothelium and ECM.

As such, there is a need for improved agents to mobilise mature leukocytes and PBC's. Surprisingly, the present inventors have discovered that low molecular weight anionic glycan mimetics induce a sustained release of circulating cells, such as mature leukocytes and HSC's, both systemically and locally. Additionally, the present inventors have discovered that these compounds do not prevent trafficking of circulating cells from the peripheral blood.

Summary

According to a first aspect of the invention, there is provided a method for mobilisation of cells capable of circulating around the body of a subject, said method comprising administration to the subject of an effective amount of low molecular weight anionic glycan mimetic, or a pharmaceutically acceptable salt thereof.

In one embodiment, the cells may be stem cells including hematopoietic stem cells, mesenchymal stem cells, bone and osteoclast stem cells, hepatic and hepatic endothelial stem cells, myogenic stem cells, endothelial stem cells, epithelial stem cells, leukocytes, platelets and erythrocytes.

The hematopoietic stem cells may be selected from the group consisting of: lymphoid (B cell, T cell and dendritic cell) progenitor cells, myeloid (basophilic, eosinophilic, neutrophilic, monocytic, mast cell, macrophage and dendritic cell) progenitor cells, platelet progenitor cells including megakaryocytes and erythroid progenitor cells including erythroblasts.

The low molecular weight anionic glycan mimetic may be selected from the group consisting of: low molecular weight heparan sulfate mimetics, low molecular weight glycosaminoglycan mimetics, monosaccharides, disaccharides, oligosaccharides, cyclic oligosaccharides (for example cyclodextrins), cyclitols, arylene ureas, pseudo sugars, and mixtures thereof, each of which may possess one or more anionic residues.

In one embodiment, the low molecular weight anionic glycan mimetic comprises one or more sulfate, phosphate or carboxylate groups.

According to a second aspect of the invention, there is provided a method for mobilising mature cells in a subject, said method comprising administration to the subject of an effective amount of a low molecular weight anionic glycan mimetic, or a pharmaceutically acceptable salt thereof.

The mature cells may be selected from the group consisting of: lymphocytes, leukocytes, platelets and erythrocytes.

The lymphocytes may be selected from the group consisting of: T cells, natural killer cells, B cells and natural killer T cells.

The leukocytes may be selected from the group consisting of: neutrophils, basophils, monocytes, eosinophils, mast cells, dendritic cells, megakaryocytes and macrophages.

According to a third aspect, the present invention provides a method for mobilising stem cells in a subject, said method comprising administration to the subject of an effective amount of a low molecular weight anionic glycan mimetic or a pharmaceutically acceptable salt thereof.

According to a fourth aspect, the present invention provides a method for the treatment of a condition in a subject where a stem cell transplant is required, said method comprising administration to the subject of a therapeutically effective amount of a low molecular weight anionic glycan mimetic, or a pharmaceutically acceptable salt thereof.

The condition may be a haematological disease, an immunologic disease or a neoplastic disease.

The condition may be selected from the group consisting of: leukemia and lymphoma, acute lymphoblastic leukemia, acute myoblastic leukemia, chronic myelogenous leukemia, Hodgkin's disease, multiple myeloma, non-Hodgkin's lymphoma, childhood brain tumors, neuroblastoma, inherited blood disorders such as aplastic anaemia, beta-thalassemia, globoid cell leukodystrophy, X-linked lymphoproliferative syndrome, severe combined immunodeficiency syndrome, inherited inborn errors of metabolism that are treated with bone marrow transplants such as Hunter's syndrome, Hurler's syndrome, fucosidosis, Lesch Nyhan syndrome, haematopoietic stem cell rescue in cancer therapy, Graft-versus-host treatment of cancer, systemic lupus erythematosus, cardiovascular disease requiring myocardial regeneration using mesenchymal stem cell transplantation, muscle degenerative diseases requiring myogenic regeneration such as muscular dystrophy, nervous system disorders requiring re-generation of neural tissue, re-myelination of nerves and glial cell repopulation such as Parkinson's disease, multiple sclerosis, Alzheimer's disease, motor neuron disease.

In accordance with the third or fourth aspects, the stem cell may be pluripotent or a hematopoetic stem cell.

The hematopoietic stem cell may be selected from the group consisting of: lymphoid (B cell, T cell and dendritic cell) progenitor cells, myeloid (basophilic, eosinophilic, neutrophilic, monocytic, mast cell, macrophage and dendritic cell) progenitor cells, platelet progenitor cells including megakaryocytes and erythroid progenitor cells including erythroblasts.

In accordance with the fourth aspect, of the invention the stem cell may be a megakaryocyte, and the condition may be selected from the group consisting of: drug-induced (e.g. heparin) thrombocytopenia, idiopathic thrombocytopenia purpura (ITP), Glanzmann thrombasthenia, Bernard-Soulier syndrome, Platelet-type von Willebrand disease, Hermansky-Pudlak and Chediak-Higashi syndromes, and defective platelet procoagulant activity (Scott syndrome).

According to a fifth aspect of the invention there is provided a composition for mobilisation of cells capable of circulating around the body of a subject, said composition comprising a low molecular weight anionic glycan mimetic, or a pharmaceutically acceptable salt thereof, and an agent, wherein the agent is capable of mobilisation of cells capable of circulating around the body of the subject.

Combinations of the low molecular weight anionic glycan mimetic and the agent may be synergistic in terms of their ability to mobilise cells capable of circulating.

The agent may be selected from the group consisting of: G-CSF, GM-CSF, IL-8, AMD-3100 and chemotherapeutic drugs, for example, methotrextrate, docetaxel, paclitaxel, epirubicin, mitoguazone, amifostine, adriamycin, taxol, fluorouracil, melphalan, cisplatin, alpha interferon, COMP (cyclophosphamide, vincristine, methotrexate and prednisone), etoposide, mBACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine and dexamethasone), PROMACE/MOPP (prednisone, methotrexate (w/leucovin rescue), doxorubicin, cyclophosphamide, taxol, etoposide/mechlorethamine, vincristine, prednisone and procarbazine), vincristine, vinblastine, angioinhibins, TNP-470, pentosan polysulfate, platelet factor 4, angiostatin, LM-609, SU-101, CM-101, Techgalan, thalidomide and SP-PG. Other chemotherapeutic agents include alkylating agents such as nitrogen mustards including mechlorethamine, melphan, chlorambucil, cyclophosphamide and ifosfamide; nitrosoureas including carmustine, lomustine, semustine and streptozocin; alkyl sulfonates including busulfan; triazines including dacarbazine; ethylenimines including thiotepa and hexamethylmelamine; folic acid analogues including methotrexate; pyrimidine analogues

including 5-fluorouracil, cytosine arabinoside; purine analogues including 6-mercaptopurine and 6-thioguanine; antitumour antibiotics including actinomycin D; the anthracyclines including doxorubicin, bleomycin, mitomycin C and methramycin; hormones and hormone antagonists including tamoxifen and corticosteroids and miscellaneous agents including cisplatin and brequinar.

In an embodiment of the invention, one or more of the mobilised cells may enter into a tissue.

Definitions

The following are some definitions that may be helpful in understanding the description of the present invention. These are intended as general definitions and should in no way limit the scope of the present invention to those terms alone, but are put forth for a better understanding of the following description.

Unless the context requires otherwise or specifically stated to the contrary, integers, steps, or elements of the invention recited herein as singular integers, steps or elements clearly encompass both singular and plural forms of the recited integers, steps or elements.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers, but not the exclusion of any other step or element or integer or group of elements or integers. Thus, in the context of this specification, the term "comprising" means "including principally, but not necessarily solely".

As used herein, the term "alkyl" includes within its meaning monovalent straight chain or branched chain saturated hydrocarbon radicals having from 1 to 10 carbon atoms, eg, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. For example, the term alkyl includes, but is not limited to, methyl, ethyl, 1-propyl, isopropyl, 1-butyl, 2-butyl, isobutyl, tert-butyl, amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, pentyl, isopentyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl, 2-ethylpentyl, 3-ethylpentyl, heptyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, 5-methylheptyl, 1-methylheptyl, octyl, nonyl, decyl, and the like.

As used herein, the term "alkenyl" includes within its meaning monovalent straight chain or branched hydrocarbon radicals having at least one double bond, and having from 2 to 10 carbon atoms, e.g. 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. For example, the term alkenyl includes, but is not limited to vinyl, propenyl, 2-methylbutenyl and hexenyl.

As used herein, the term "alkoxy" refers to o-alkyl, where alkyl is as defined above.

As used herein the term "halo" includes within its meaning fluoro, chloro, bromo and iodo.

As used herein, the term "aryl" includes within its meaning monovalent, single, polynuclear, conjugated and fused aromatic hydrocarbon radicals, for example phenyl, naphthyl, anthracenyl, pyrenyl, phenanthracenyl.

As used herein, the term "heteroaryl" includes within its meaning monovalent, single, polynuclear conjugated and fused aromatic radicals having 1 to 15 carbons wherein 1 to 6 atoms are hetero atoms selected from O, N and S.

As used herein the term "arylene" includes within its meaning divalent, single, polynuclear, conjugated and fused aromatic hydrocarbon radicals.

As used herein the term "cyclitol" includes within its meaning cycloalkanes comprising one hydroxyl group on each of three or more ring atoms.

As used herein the term "pseudo sugar" includes within its meaning monosaccharide, disaccharide or oligosaccharide molecules in which one or more of the "saccharide" units do not comprise an oxygen atom.

In the context of this specification the term "administering" and variations of that term including "administer" and "administration", includes contacting, applying, delivering or providing a compound or composition of the invention to an organism by any appropriate means.

In the context of this specification, the term "treatment", refers to any and all uses which remedy a disease state or symptoms, prevent the establishment of disease, or otherwise prevent, hinder, retard, or reverse the progression of disease or other undesirable symptoms in any way whatsoever.

In the context of this specification the term "effective amount" includes within its meaning a sufficient but non-toxic amount of a compound or composition of the invention to provide the desired effect. The exact amount required will vary from subject to subject depending on factors such as the desired effect, the species being treated, the age and general condition of the subject, the severity of the condition being treated, the particular agent being administered, the mode of administration, and so forth. Thus, it is not possible to specify an exact "effective amount". However, for any given case, an

appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

In the context of the present specification the term "C21" refers to the product obtained following the reaction of lactobionic acid with pyridine-sulfur trioxide complex as described in Example 1.

In the context of the present specification, "low molecular weight anionic glycan mimetic" refers to sugar or saccharide mimetics or analogues or sugar-like compounds having molecular weights less than about 5kDa.

In the context of the present specification the terms "ring-opened monosaccharide", "ring-opened disaccharide" and "ring-opened oligosaccharide" refer to the respective saccharide molecules wherein at least one ring is present in the open chain form. The "ring-opened" compound may be for example an alditol or a glycol split, or any other product of complete or partial oxidation and/or reduction of said monosaccharide, disaccharide or oligosaccharide arising from, for example, reactions as are known in the art such as sodium borohydride reduction.

In the context of the present specification, the terms "mobilisation" and "mobilising" refer to the migration of cells from a position where the cells were initially localised or stored (for example in the bone marrow, tissues or lymph tissue) and into circulation.

In the context of the present specification, the terms "progenitor cell" and "stem cell" are used interchangeably and should be understood to have the same meaning.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

Brief Description of the Drawings

A preferred form of the present invention will now be described by way of examples with reference to the accompanying drawings wherein:

Figure 1 shows various routes of administration (intravenous, intranasal and intraperitoneal) used to monitor the comparative effects of C21-induced eosinophilia.

Figure 2A shows a leukocyte profile in the peripheral blood following intravenous treatment with C21 (50μg/mouse). Each cell type was measured as a percentage of the total leukocyte population in the peripheral blood.

Figure 2B shows an eosinophil profile in the peripheral blood following intravenous treatment with C21 (50μg/mouse). Eosinophils were measured as a percentage of the total leukocyte population in the peripheral blood.

Figure 2C shows neutrophilia, eosinophilia and progenitor presence in blood smears following intravenous C21 treatment.

Figures 3A, 3B and 3C show leukocyte, eosinophil and neutrophil profiles in the peripheral blood respectively, following intravenous treatment with high dose (1mg/mouse) C21. Each cell type was measured as a percentage of the total leukocyte population in the peripheral blood.

Figure 4A shows an eosinophil profile in peripheral blood over a period of 6 hours following treatment of mice with various sulfated oligosaccharide derivatives. Eosinophils were measured as a percentage of the total leukocyte population in the peripheral blood.

Figure 4B shows a neutrophil profile in peripheral blood over a period of 6 hours following treatment of mice with various sulfated oligosaccharides. Neutrophils were measured as a percentage of the total leukocyte population in the peripheral blood.

Figure 4C shows a leukocyte profile in peripheral blood following treatment of mice with various oligosaccharides after 0 hours. Each cell type was measured as a percentage of the total leukocyte population in the peripheral blood.

Figure 4D shows a leukocyte profile in peripheral blood following treatment of mice with various oligosaccharides after 2 hours. Each cell type was measured as a percentage of the total leukocyte population in the peripheral blood.

Figure 4E shows a leukocyte profile in peripheral blood following treatment of mice with various oligosaccharides after 6 hours. Each cell type was measured as a percentage of the total leukocyte population in the peripheral blood.

Figure 5 shows the induction of leukocytosis and SDF-1α release into the peripheral blood following administration of synthetically-made sulfated glycan mimetics (SSGMs).

Figure 6A shows the effect of maltopentaose sulfate (MPS), C21 and fucoidan treatment on leukocyte trafficking in the peripheral blood. Total leukocytes in peripheral blood were calculated by manual haemocytometer counts following methylene blue staining.

Figure 6B shows the effect of maltopentaose sulfate (MPS), C21 and fucoidan treatment on neutrophil trafficking in the peripheral blood. Total neutrophils in peripheral blood were calculated by comparing differential cell counts to total leukocyte counts.

Figure 6C shows the effect of maltopentaose sulfate (MPS), C21 and fucoidan treatment on EGFP+ cell trafficking in the peripheral blood. The percentage of total leukocytes found to be EGFP+ in peripheral blood was determined by FACS analysis.

Figure 6D shows the effect of maltopentaose sulfate (MPS), C21 and fucoidan treatment on EGFP+ cell trafficking in the spleen. The percentage of total leukocytes found to be EGFP+ in the spleen was determined by FACS analysis.

Figure 7A shows an optical biosensor analysis of the effect of sulfated oligosaccharides on the association between heparin and eotaxin, and identifies C21 as an inhibitor of eotaxin/heparin association.

Figure 7B shows the effect of SSGMs on eotaxin binding to immobilised heparin.

Figure 8A shows haematopoietic progenitor cell (HPC) mobilisation after treatment with cyclophosphamide +/- fucoidan, maltopentaose sulfate or C21.

Figure 8B shows a quantitative analysis of haematopoietic progenitor cell (HPC) mobilisation after treatment with cyclophosphamide +/- fucoidan, maltopentaose sulfate or C21, indicated by the percentage of $Lin^-c\text{-}Kit^+$ cells in total peripheral blood.

Figure 9A shows haematopoietic stem cell (HSC) mobilisation after treatment with cyclophosphamide +/- fucoidan, maltopentaose sulfate or C21.

Figure 9B shows a quantitative analysis of haematopoietic stem cell (HSC) mobilisation after treatment with cyclophosphamide +/- fucoidan, maltopentaose sulfate or C21, indicated by the percentage of $Lin^-c\text{-}Kit^+Sca^+$ cells in total peripheral blood.

Figure 9C shows a quantitative analysis of haematopoietic stem cell (HSC) mobilisation after treatment with cyclophosphamide +/- fucoidan, maltopentaose sulfate or C21, indicated by the percentage of $Lin^-c\text{-}Kit^+Sca^+$ cells as a portion of total $Lin^-c\text{-}Kit+$ cells in peripheral blood.

Detailed Description

The present invention is directed to a method for mobilisation of cells that are capable of circulating around the body of a subject. The method comprises administration to the subject of a low-molecular weight anionic glycan mimetic.

The low molecular weight anionic glycan mimetic may be selected from the group consisting of: a monosaccharide, a disaccharide, an oligosaccharide, a cyclic

oligosaccharide (for example a cyclodextrin), a cyclitol, an arylene urea comprising one or more anionic residues, a pseudo sugar, and mixtures thereof.

In one embodiment, the monosaccharide is a sulfated monosaccharide, the disaccharide is a sulfated disaccharide and the oligosaccharide is a sulfated oligosaccharide.

In one embodiment, the monosaccharide may be a ring-opened monosaccharide, the disaccharide may be a ring-opened disaccharide, and the oligosaccharide may be a ring-opened oligosaccharide.

In another embodiment, the low molecular weight anionic glycan mimetic is a monosaccharide, disaccharide, oligosaccharide, ring-opended monosaccharide, ring-opended disaccharide or ring-opended oligosaccharide having the following structural formula:

$A\text{-}(B)_a$

wherein a is an integer between 0 and 9; A is selected from the group consisting of: a diose, a triose, a tetraose, a pentose, a hexose, a heptose, an octose and a nonose, and each independent B is selected from the group consisting of: a diose, a triose, a tetraose, a pentose, a hexose, a heptose, an octose and a nonose;

wherein A and B, and where a is an integer of 2 or greater, B and B, linked via a group selected from: $-O\text{-}(CH_2)_x\text{-}O-$, -O-, $-OCH_2-$, -NH-, -S-, $-NR(CH_2)_x\text{-}Ar\text{-}(CH_2)_xNR_1-$, $-NR(CH_2)_xNR_1-$, $-O(CH_2)_x\text{-}Ar\text{-}(CH_2)_xO-$, $-C(O)\text{-}N(R_2)\text{-}(CH_2)_x\text{-}N(R_2)\text{-}C(O)-$, $-N(R_2)\text{-}C(O)\text{-}Ar\text{-}(CH_2)_x\text{-}Ar\text{-}C(O)\text{-}N(R_2)-$ and $-N(R_2)\text{-}(CH_2)_x\text{-}N(R_2)-$; R, R_1 and R_2 are selected from the group consisting of: hydrogen, alkyl, aryl, heteroaryl and C(O)-alkyl;

x is an integer between 0 and 10;

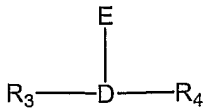
wherein A and B may be substituted with a functional group selected from the group consisting of: alkyl, alkenyl, aryl, halo, heteroaryl, an amide derivative such as $-NHCOCH_3-$, alkoxy such as $-OCH_3-$, -O- and -OH-;

and wherein said diose, triose, tetraose, pentose, hexose, heptose, octose and nonose may be sulfated, phosphorylated or carboxylated.

In an embodiment of the first aspect, A and each B are independently selected from the group consisting of a pentose, a hexose and a heptose, and are linked via a group selected from: $-O\text{-}(CH_2)_x\text{-}O-$, -O-, $-OCH_2-$, $-NR(CH_2)_x\text{-}Ar\text{-}(CH_2)_xNR_1-$, $-O(CH_2)_x\text{-}Ar\text{-}(CH_2)_xO-$, $-C(O)\text{-}N(R_2)\text{-}(CH_2)_x\text{-}N(R_2)\text{-}C(O)-$, $-N(R_2)\text{-}C(O)\text{-}Ar\text{-}(CH_2)_x\text{-}Ar\text{-}C(O)\text{-}N(R_2)-$, and R, R_1 and R_2 are selected from the group consisting of: hydrogen, acetyl and alkyl, and x is an integer between 1 and 6.

In another embodiment of the first aspect, the hexose may be selected from the group consisting of: glucose, galactose, mannose, fructose, fucose, and idose, and the pentose may be xylose.

In a further embodiment of the first aspect, the low molecular weight anionic glycan mimetic is a cyclitol having the following structural formula:



wherein:

D is selected from the group consisting of: N, CH, O, S, or a linker selected from -CO-NH-G-NH-CO-, -NH-CO-G-CO-NH-, -NH-G-NH-, -O-G-O-;

G is selected from the group consisting of alkylene and arylene;

R_3 is a 4-, 5-, or 6- membered carbocyclic ring that is saturated or unsaturated, wherein the ring comprises at least one sulfate group, at least one carboxylate group or at least one phosphate group.

R_4, is selected from the group consisting of: a 4-, 5-, or 6- membered carbocyclic ring that is saturated or unsaturated, wherein the ring comprises at least one sulfate group, at least one carboxylate group or at least one phosphate group, hydrogen, aryl and alkyl;

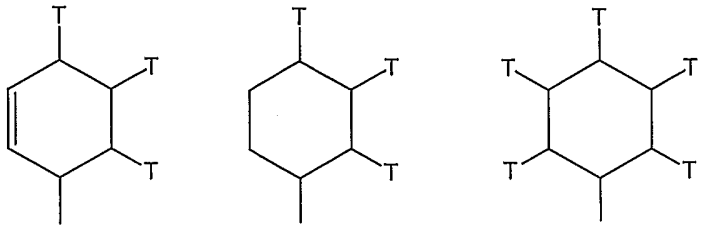
E is selected from the group consisting of: hydrogen, alkyl, aryl, -B-C(R_5)(R_6) and acetate;

B is selected from the group consisting of: -$(CH_2)_x$-, -$CH_2C_6H_4CH_2$-, -$CH_2CH(OH)CH_2$-, -$(CH_2)_x$-Ar-$(CH_2)_x$-, wherein the B group may optionally comprise one or more sulfate groups, one or more carboxylate groups or one or more phosphate groups.

R_5 and R_6 are independently selected from the group consisting of: 4-, 5-, or 6- membered carbocyclic ring that is saturated or unsaturated, hydrogen, aryl and alkyl, wherein R_5 and/or R_6 may comprise one or more sulfate groups, one or more carboxlyate groups or one or more phosphate groups, and x is an integer between 0 and 10.

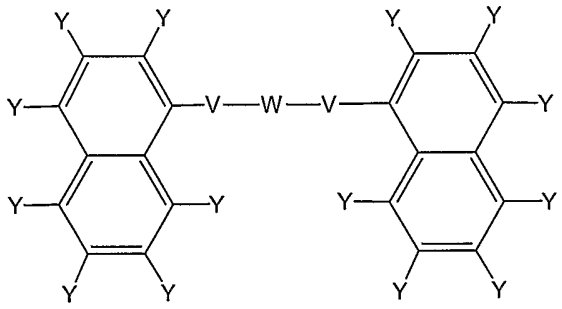
In one embodiment, B is selected from the group consisting of: -$(CH_2)_x$-, wherein x is an integer between 2 and 10, $CH_2C_6H_4CH_2$ and $CH_2CH(OSO_3H)CH_2$.

In an alternative embodiment, R_3, R_4, R_5 and R_6 may be independently selected from the following:



wherein T is independently selected from the group consisting of: SO_3H, SO_3^-, COOH, COO^-, OPO_3H and OPO_3^-.

In a further embodiment of the first aspect, the low molecular weight anionic glycan mimetic is an arylene urea of the following formula:



wherein each Y is independently selected from the group consisting of: SO_3H, SO_3^-, hydrogen, alkyl, halo, phenyl, an amide derivative, $-NHCOCH_3$, -O-, $-OCH_3$, COOH, COO^-, OPO_3H and OPO_3^-.

each V is independently selected from the group consisting of: $-(NHC(O)Ph)_z-$, $(CH_2)_u$ and phenyl;

W is -NH-C(O)-NH-;

u and z may independently of each other be an integer between 0 and 10.

In one embodiment, the arylene urea may be suramin, or a salt thereof.

In another embodiment of the invention, the glycosaminoglycan mimetic may be a sulfated cyclic oligosaccharide, wherein the oligosaccharide is cyclodextrin.

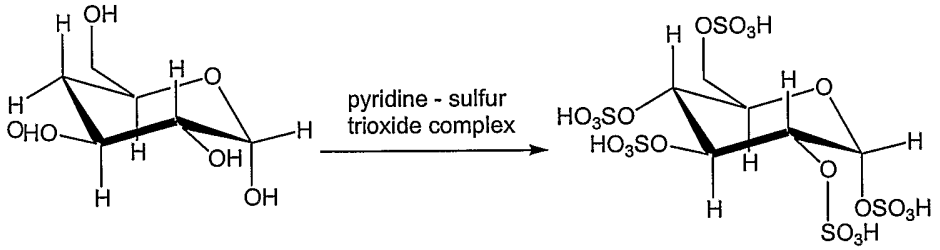
In a further embodiment of the invention the low molecular weight anionic glycan mimetic is aprosulate.

Synthesis of compounds

Low molecular weight anionic glycan mimetics for use in the compositions and methods of the invention may be purchased or prepared by methods known to those skilled in the art.

Sulfated saccharide compounds used in the methods and compositions of the invention may be prepared by sulfation of a corresponding monosaccharide, disaccharide or oligosaccharide also by methods known to those skilled in the art. For example, the

saccharide compound may be treated with a sulfating agent such as pyridine-sulfur trioxide complex in the presence of an appropriate solvent as follows:

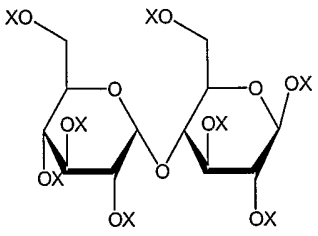


In one aspect of the invention, the low molecular anionic glycan mimetic may be a mixture of compounds obtained by reaction of a monosaccharide, disaccharide or oligosaccharide with pyridine-sulfur trioxide complex.

The low molecular weight anionic glycan mimetics may have one or more sulfate groups present. These sulfate groups may react with various bases to form salts. The sulfated compounds are stable when in the form of a salt. The sulfated compounds in a free form may be derived from a salt thereof by utilizing a cation-exchange resin such as Dowex 50W-X8. Optionally, a salt can be subjected to conventional ion-exchange to convert it into any one of various other desirable salts.

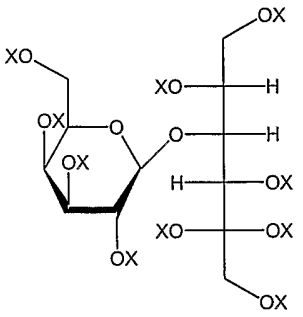
The oligosaccharides that are sulfated may be naturally occurring products, for example raffinose, stachyose or cyclodextrins. Alternatively, the oligosaccharides may be prepared by enzymatic or chemical degradation of naturally occurring polysaccharides, followed by subsequent chemical modification.

Preferred low molecular weight anionic glycan mimetics useful in the methods and compositions of the invention include the following:

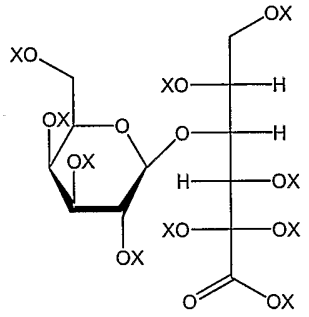


Maltose C10

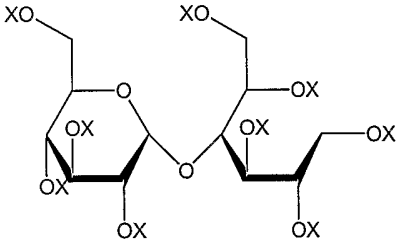
Wherein X = H or SO_3H



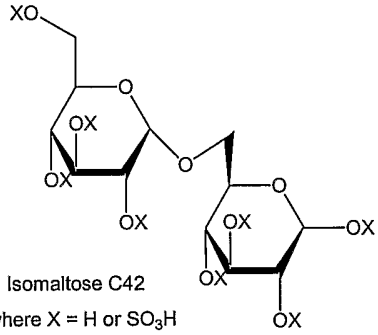
Lactitol C18
wherein X = H or SO_3H



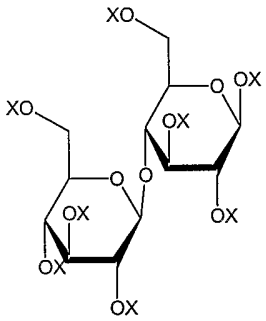
Lactobionic Acid
wherein X = H or SO_3H



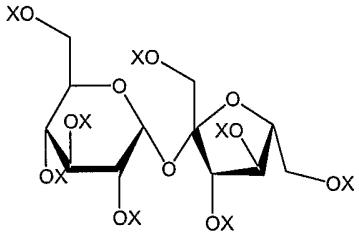
Maltitol C22
wherein X = H or SO_3H



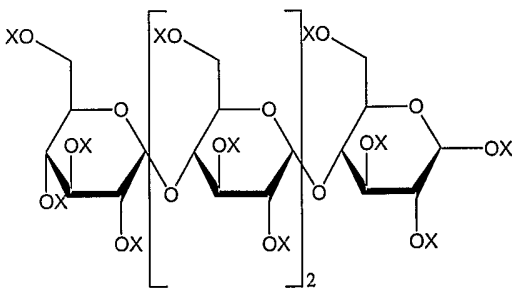
Isomaltose C42
where X = H or SO_3H



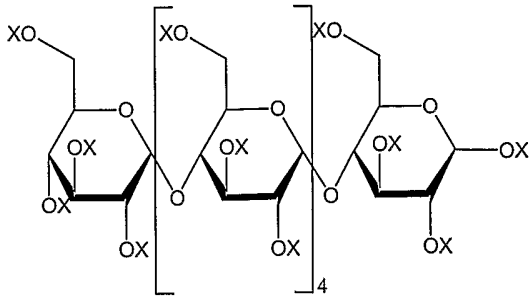
Cellobiose
Where X = H or SO_3H



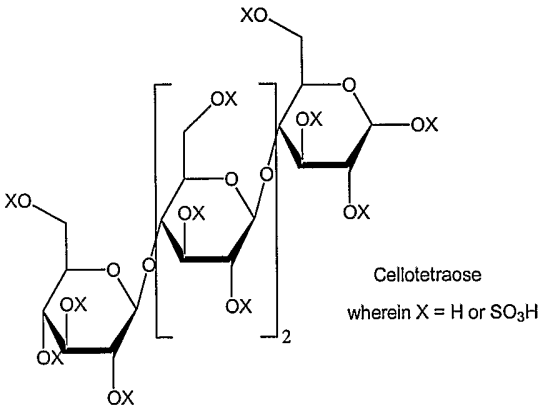
Sucrose
where X = H or SO_3H

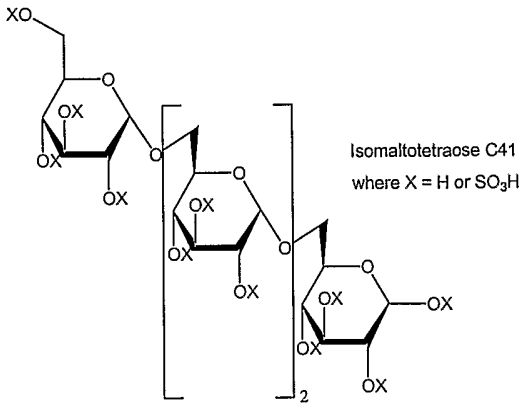


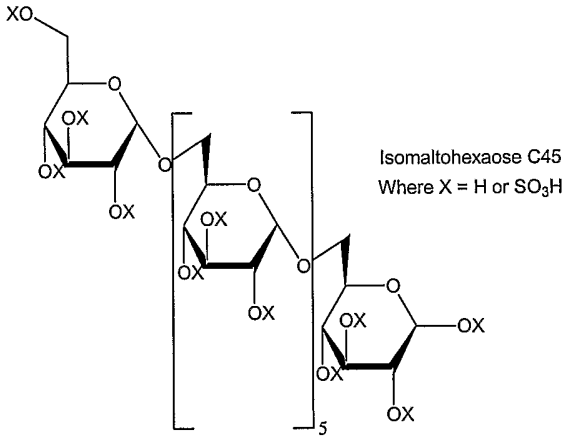
Maltotetraose C14
wherein X = H or SO_3H

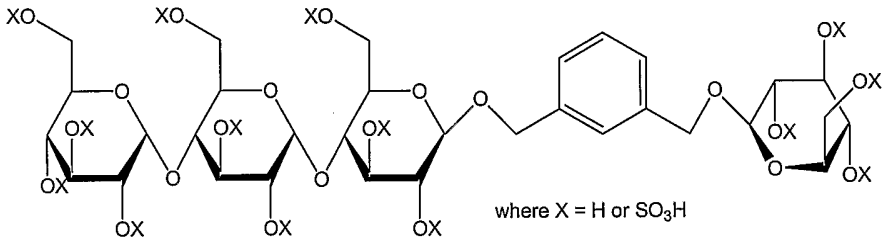


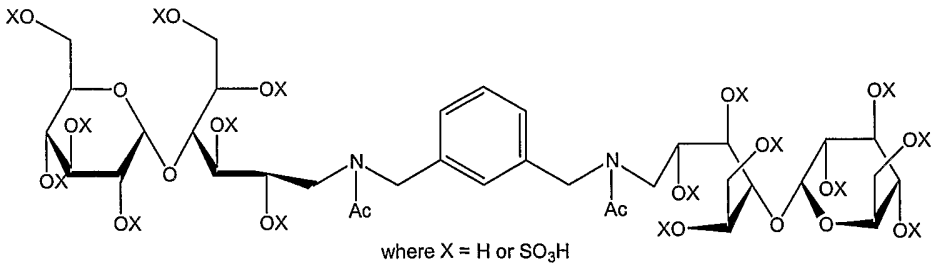
Maltohexaose C46
Where X = H or SO_3H

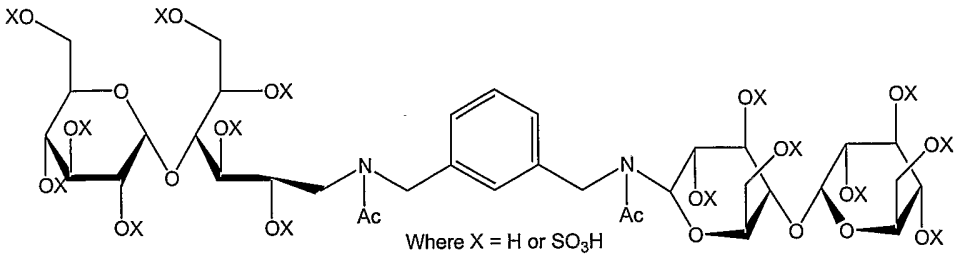

Cellotetraose
wherein X = H or SO_3H

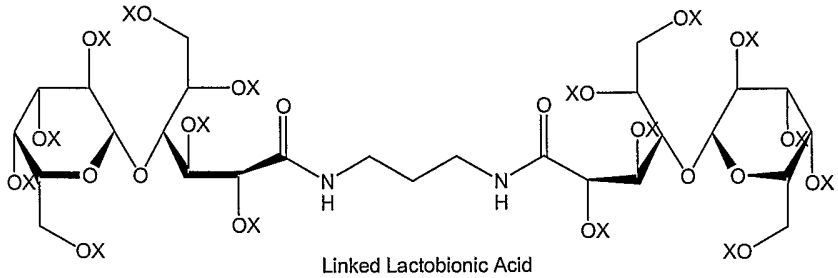

Isomaltotetraose C41
where X = H or SO_3H

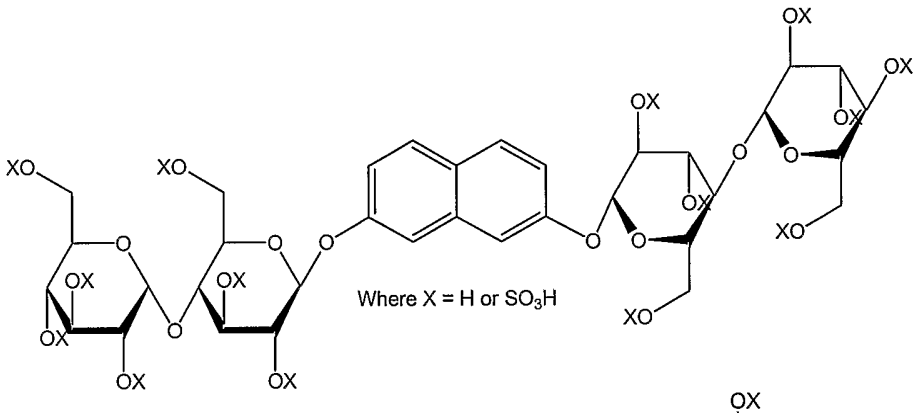

Isomaltohexaose C45
Where X = H or SO_3H

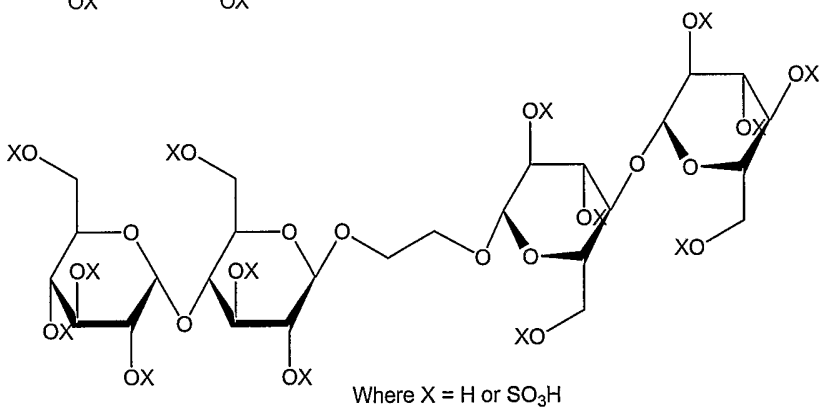

where X = H or SO_3H

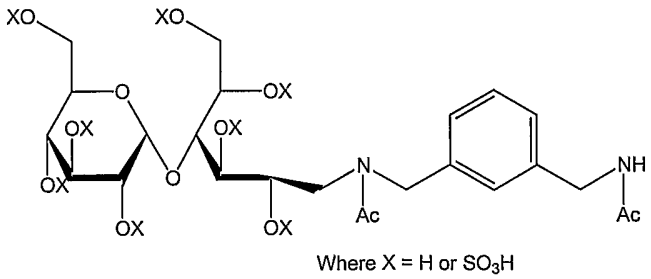

where X = H or SO_3H

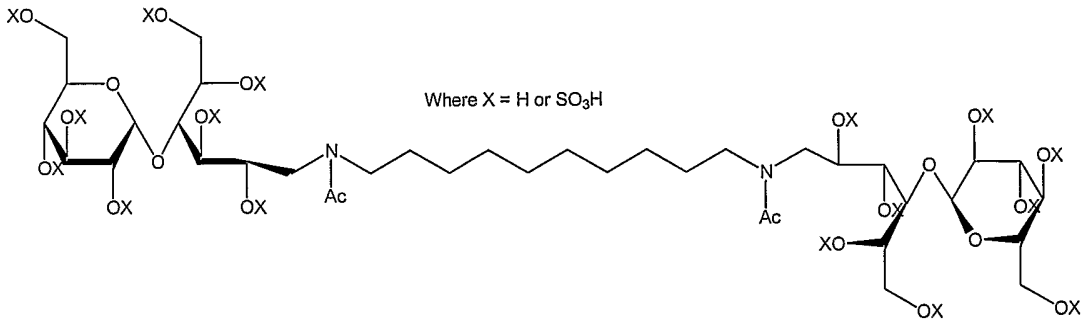

Where X = H or SO_3H

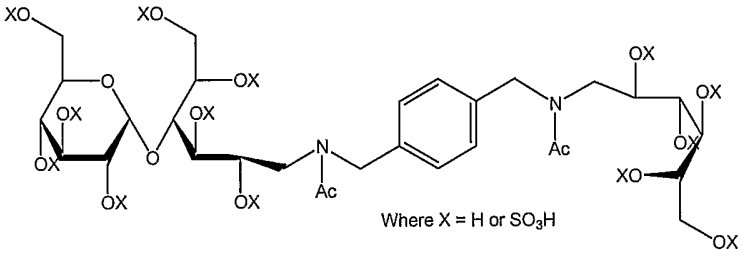

Linked Lactobionic Acid
where X = H or SO_3H

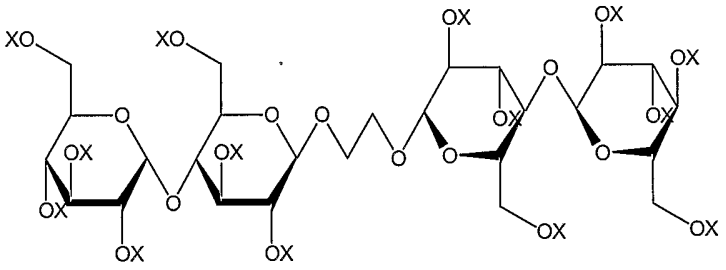

Where X = H or SO_3H

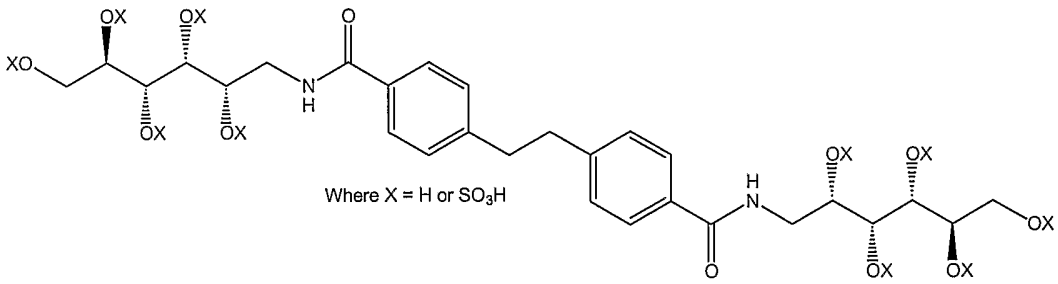

Where X = H or SO_3H

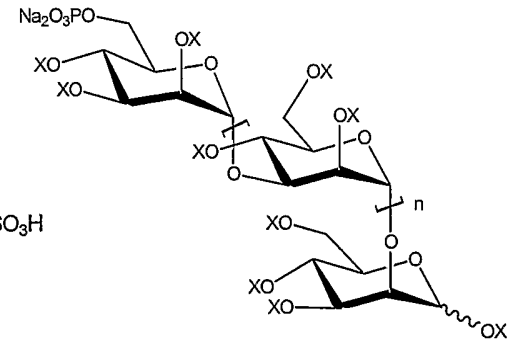

Where X = H or SO_3H











PI-88

where X = H or SO_3H
n is 0-4

The second and third aspects of the invention respectively provide methods for mobilising leukocytes and stem cells in a subject. The methods comprise administration

to the subject of an effective amount of a low molecular weight anionic glycan mimetic, or a pharmaceutically acceptable salt thereof.

The anionic glycan mimetic may be as defined in the first aspect of the invention.

In general, compounds capable of mobilising stem cells are useful in the treatment of conditions where a stem cell transplant is required. As such, the present invention is also useful in the treatment of conditions in a subject where a stem cell transplant is required.

Accordingly, in a fourth aspect the present invention provides a method for the treatment of a condition in a subject where a stem cell transplant is required, said method comprising administration of a therapeutically effective amount of a low molecular weight glycosaminoglycan mimetic, or a pharmaceutically acceptable salt thereof.

According to a fifth aspect of the invention there is provided a composition for mobilisation of cells capable of circulating around the body of a subject, said composition comprising a low molecular weight glycosaminoglycan mimetic, or a pharmaceutically acceptable salt thereof, and an agent, wherein the agent is capable of mobilisation of cells capable of circulating around the body of the subject.

The anionic glycan mimetic and the agent may be administered simultaneously, sequentially or in succession. Where the anionic glycan mimetic and the agent are administered simultaneously, they may be present in a single composition which comprises both the glycosaminoglycan mimetic and the agent, or alternatively the anionic glycan mimetic and the agent may be present in separate compositions, one comprising the anionic glycan mimetic and one comprising the agent, which are administered simultaneously.

Examples of conditions which may be treated using a stem cell transplant may be selected from, but not limited to, the group consisting of: allogenic autologous leukemias, for example acute myelogenous, acute lymphoblastic, chronic myelogenous, chronic lymphocytic; lymphomas, for example Hodgkin's and non-Hodgkins; plasma cell disorders, for example, myeloma and amyloidosis; solid tumours, for example breast cancer, ovarian cancer, testicular cancer, renal cell cancer, brain tumours, neuroblastoma, Ewing's sarcoma; acquired bone marrow disorders, for example severe aplastic anemia, myelodysplastic syndrome, myeloproliferative disorders; congenital disorders, for example immunodeficiencies, Wiskott Aldrich's, Fanconi's anemia, Thalassemia, sickle cell anemia, osteoperosis and storage diseases; auto-immune diseases, for example scleroderma, rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis.

Compositions and routes of administration

The low molecular weight anionic glycan mimetics may be present as pharmaceutically acceptable salts. By "pharmaceutically acceptable salt", it is meant those salts which, within the scope of sound medical judgement, are suitable for use in contact with tissues of humans and lower animals without the undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art.

The therapeutically effective amount of the compounds or agents disclosed herein for any particular subject will depend upon a variety of factors including: the disorder being treated and the severity of the disorder; activity of the compositions employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the route of administration; the rate of sequestration of the compositions; the duration of the treatment; drugs used in combination or coincidental with the treatment, together with other related factors well known in medicine.

One skilled in the art would be able, by routine experimentation, to determine an effective, non-toxic amount of the components of the formulations which would be required to treat applicable to achieve the desired outcome of the methods of the invention.

Generally, an effective dosage of the low molecular weight anionic glycan mimetic is expected to be in the range of about 0.0001mg to about 1000mg per kg body weight per 24 hours; typically, about 0.001mg to about 750mg per kg body weight per 24 hours; about 0.01mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 250mg per kg body weight per 24 hours; about 1.0mg to about 250mg per kg body weight per 24 hours. More typically, an effective dose range is expected to be in the range about 1.0mg to about 200mg per kg body weight per 24 hours; about 1.0mg to about 100mg per kg body weight per 24 hours; about 1.0mg to about 50mg per kg body weight per 24 hours; about 1.0mg to about 25mg per kg body weight per 24 hours; about 5.0mg to about 50mg per kg body weight per 24 hours; about 5.0mg to about 20mg per kg body weight per 24 hours; about 5.0mg to about 15mg per kg body weight per 24 hours.

Alternatively, an effective dosage of a low molecular weight anionic glycan mimetic may be up to about 500mg/m^2. Generally, an effective dosage is expected to be in the range of about 25 to about 500mg/m^2, preferably about 25 to about 350mg/m^2, more preferably about 25 to about 300mg/m^2, still more preferably about 25 to about 250mg/m^2, even more preferably about 50 to about 250mg/m^2, and still even more preferably about 75 to about 150mg/m^2.

Generally, an effective dosage of the agent is expected to be in the range of about 0.0001mg to about 1000mg per kg body weight per 24 hours; typically, about 0.001mg to about 750mg per kg body weight per 24 hours; about 0.01mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 250mg per kg body weight per 24 hours; about 1.0mg to about 250mg per kg body weight per 24 hours. More typically, an effective dose range is expected to be in the range about 1.0mg to about 200mg per kg body weight per 24 hours; about 1.0mg to about 100mg per kg body weight per 24 hours; about 1.0mg to about 50mg per kg body weight per 24 hours; about 1.0mg to about 25mg per kg body weight per 24 hours; about 5.0mg to about 50mg per kg body weight per 24 hours; about 5.0mg to about 20mg per kg body weight per 24 hours; about 5.0mg to about 15mg per kg body weight per 24 hours.

Alternatively, an effective dosage of the agent may be up to about 500mg/m^2. Generally, an effective dosage is expected to be in the range of about 25 to about 500mg/m^2, preferably about 25 to about 350mg/m^2, more preferably about 25 to about 300mg/m^2, still more preferably about 25 to about 250mg/m^2, even more preferably about 50 to about 250mg/m^2, and still even more preferably about 75 to about 150mg/m^2.

Further, it will be apparent to one of ordinary skill in the art that the optimal quantity and spacing of individual dosages will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the nature of the particular individual being treated. Also, such optimum conditions can be determined by conventional techniques.

It will also be apparent to one of ordinary skill in the art that the optimal course of treatment, such as, the number of doses of the composition given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

In general, suitable compositions may be prepared according to methods which are known to those of ordinary skill in the art and accordingly may include a pharmaceutically acceptable carrier, diluent and/or adjuvant.

Convenient modes of administration include injection (subcutaneous, intravenous, etc.), oral administration, intranasal, inhalation, transdermal application, topical creams or gels or powders, or rectal administration. Depending on the route of administration, the formulation and/or compound may be coated with a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the therapeutic activity of the compound. The compound may also be administered parenterally or intraperitoneally.

Dispersions of compounds may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, pharmaceutical preparations may contain a preservative to prevent the growth of microorganisms.

Pharmaceutical compositions suitable for injection include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Ideally, the composition is stable under the conditions of manufacture and storage and may include a preservative to stabilise the composition against the contaminating action of microorganisms such as bacteria and fungi.

In one embodiment of the invention, the compound(s) may be administered orally, for example, with an inert diluent or an assimilable edible carrier. The compound(s) and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into an individual's diet. For oral therapeutic administration, the compound(s) may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Suitably, such compositions and preparations may contain at least 1% by weight of active compound. The percentage of the anionic glycan mimetic in pharmaceutical compositions and preparations may, of course, be varied and, for example, may conveniently range from about 2% to about 90%, about 5% to about 80%, about 10% to about 75%, about 15% to about 65%; about 20% to about 60%, about 25% to about 50%, about 30% to about 45%, or about 35% to about 45%, of the weight of the dosage unit. The amount of compound in therapeutically useful compositions is such that a suitable dosage will be obtained.

The language "pharmaceutically acceptable carrier" is intended to include solvents, dispersion media, coatings, anti-bacterial and anti-fungal agents, isotonic and absorption delaying agents, and the like. Examples of pharmaceutically acceptable carriers or diluents are demineralised or distilled water; saline solution; vegetable based oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oil, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysolpoxane; volatile silicones; mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose or hydroxypropylmethylcellulose; lower alkanols, for example ethanol or iso-propanol;

lower aralkanols; lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerin; fatty acid esters such as isopropyl palmitate, isopropyl myristate or ethyl oleate; polyvinylpyrridone; agar; carrageenan; gum tragacanth or gum acacia, and petroleum jelly. Typically, the carrier or carriers will form from 10% to 99.9% by weight of the compositions.

The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the compound, use thereof in the therapeutic compositions and methods of treatment and prophylaxis is contemplated. Supplementary active compounds may also be incorporated into the compositions according to the present invention. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. "Dosage unit form" as used herein refers to physically discrete units suited as unitary dosages for the individual to be treated; each unit containing a predetermined quantity of compound(s) is calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The compound(s) may be formulated for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in an acceptable dosage unit. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

In one embodiment, the carrier may be an orally administrable carrier.

Another form of a pharmaceutical composition is a dosage form formulated as enterically coated granules, tablets or capsules suitable for oral administration.

Also included in the scope of this invention are delayed release formulations.

Compounds may also be administered in the form of a "prodrug". A prodrug is an inactive form of a compound which is transformed *in vivo* to the active form. Suitable prodrugs include esters, phosphonate esters etc, of the active form of the compound.

Some examples of suitable carriers, diluents, excipients and adjuvants for oral use include peanut oil, liquid paraffin, sodium carboxymethylcellulose, methylcellulose, sodium alginate, gum acacia, gum tragacanth, dextrose, sucrose, sorbitol, mannitol, gelatine and lecithin. In addition these oral formulations may contain suitable flavouring and colourings agents. When used in capsule form the capsules may be coated with compounds such as glyceryl monostearate or glyceryl distearate which delay disintegration.

In one embodiment, the compound may be administered by injection. In the case of injectable solutions, the carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by including various anti-bacterial and/or anti-fungal agents. Suitable agents are well known to those skilled in the art and include, for example, parabens, chlorobutanol, phenol, benzyl alcohol, ascorbic acid, thimerosal, and the like. In many cases, it may be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilisation. Generally, dispersions are prepared by incorporating the analogue into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above.

Tablets, troches, pills, capsules and the like can also contain the following: a binder such as gum gragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it can contain, in addition to materials of the above type, a liquid carrier. Various other materials can be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules can be coated with shellac, sugar or both. A syrup or elixir can contain the analogue, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the analogue can be incorporated into sustained-release preparations and formulations.

The pharmaceutical compositions may further include a suitable buffer to minimise acid hydrolysis. Suitable buffer agent agents are well known to those skilled in the art and include, but are not limited to, phosphates, citrates, carbonates and mixtures thereof.

Single or multiple administrations of the pharmaceutical compositions according to the invention may be carried out. One skilled in the art would be able, by routine experimentation, to determine effective, non-toxic dosage levels of the compound and/or composition of the invention and an administration pattern which would be suitable for treating the diseases and/or infections to which the compounds and compositions are applicable.

Further, it will be apparent to one of ordinary skill in the art that the optimal course of treatment, such as the number of doses of the compound or composition of the invention given per day for a defined number of days, can be ascertained using convention course of treatment determination tests.

Examples

Example 1 - Preparation of sulfated oligosaccharides

Synthesis

200 mg of oligosaccharide (e.g. the disaccharide lactobionic acid) was dissolved in 10 mL of anhydrous *N,N*-dimethyl formamide (DMF) (Aldrich, WI, USA). 3.2 g of pyridine-sulfur trioxide complex (Aldrich, MO, USA), a ten-fold excess over the number of free hydroxyl groups in the oligosaccharide, was added to the mixture. The vessel was heated at 60°C and stirred for 6 hours. The cooled supernatant was decanted and the residue was washed twice with 10 mL methanol. The residue was dissolved in the 10 mL of water and neutralised with sodium hydroxide. The solution was applied to a column (1.5 x 30 cm) of DOWEX 50W-X8 cation exchange resin (Na^+ form; Bio-Rad Laboratories, Hercules, CA). The column was developed with water and each fraction tested for sulfate content by dimethylene blue assay (see below). The sulfate-containing fractions were combined and dialysed in a 500 Da membrane sack (Spectropore) in 5 L of water at 4°C for 36 hours, with 4 changes of water. The contents of the sack were lyophilised, and stored in powder form in a dessicator at 4°C.

Using the above method, for some oligosaccharide reagents, no residue formed during the initial reaction. To the DMF solution was added 20 mL of methanol and the solution kept at -20°C overnight. The supernatant was decanted and any residue that had

precipitated out of these methanol washings was dissolved in 10 mL of water, neutralised and treated as above.

Dimethylene blue assay

100 µL of 500 µg/mL solution of sulfated oligosaccharide was added to the first well of a 96 well plate (Nunclon Nunc, Roskilde, Denmark) and serially diluted 1 in 2 across the plate starting with 500 µg/mL. 250 µL of 16 µg/mL dimethylene blue (Sigma, MO, CA) was added to each well and mixed thoroughly. The absorbance of each well at 650 nm was measured using the Thermomax microplate reader (Molecular Devices, GMI, Ramsey, USA).

Example 2: Isolation and analysis of peripheral blood (PB)

Mice

Male BALB/c wild type mice were obtained from specific pathogen free facilities at the John Curtin School of Medical Research (JCSMR) and housed in approved containment facilities at the Australian National University (ANU). Mice were treated in accordance with ANU animal experimentation guidelines. Mice used in experiments were aged between 6 and 8 weeks.

Peripheral Blood

Peripheral blood smears were taken from the tail vein. The blood smears were stained with May-Grünwald Geimsa stain and 200-300 cells were counted in each smear and differentiated on the basis of morphological characteristics to determine the percentage of cells in the leukocyte population of the peripheral blood.

Example 3: Measurement of the induction of eosinophilia using different routes of C-21 administration

Mice were administered C21 (50µg/mouse) once by intravenous, intraperitoneal, or intranasal administration. To intranasally administer C21, mice were anaesthetised with isofluorane aerosol and 50 µL of C21 then applied dropwise to the nostrils. Control mice were not administered C21. Blood smears from the tail vein were taken 0, 12, 24, 36 and 48 hours post C21 challenge. An eosinophil profile in the peripheral blood was generated according to methods outlined in Example 2. No significant difference was observed between the levels of eosinophilia generated by the intravenous or intranasal

administration of 50μg/mouse of C21, however there was a reduction in eosinophils induced by the intraperitoneal route (Figure 1). Peripheral blood eosinophilia via the three routes of administration was maximal at 24 hours at the 50μg/mouse dose (Figure 1).

Example 4: Leukocyte profiling in peripheral blood following C21 challenge

Mice were administered C21 (50μg/mouse) once by intravenous injection. Control mice were not administered C21. Blood smears from the tail vein were taken 0, 0.5, 2, 6 and 24 hours post C21 challenge. The leukocyte profile of the peripheral blood was investigated at each timepoint. Neutrophil levels were elevated from 18% to 52% by 0.5 hours post injection in the peripheral blood, accompanied by a corresponding decrease in the percentage of lymphocytes (Figure 2A).

Peripheral blood eosinophilia rose significantly from 2.5% to 9.5% by 0.5 hours post injection, remaining high at the 24hr completion of the experiment (Figure 2B).

Throughout the leukocyte profiling process it became clear that a large proportion of the cells were proving difficult to differentiate. Further investigation suggested the release of neutrophilic, eosinophilic, lymphocytic and monocytic stem cell progenitors in response to C21 intravenous treatment (Figure 2C).

Example 5: Comparison of C21- or fucoidan-induced leukocytosis

Mice were administered C21 or fucoidan (1mg/mouse or 50mg/kg) once by intravenous injection. Blood smears from the tail vein were taken 0, 0.5, 2, and 6 hours post C21 or fucoidan challenge. The leukocyte profile of the peripheral blood was compared following intravenous treatment with C21 or fucoidan at each timepoint.

A 4-5 fold leukocytosis (increase in leukocytes) was observed following treatment with C21 and fucoidan (Figure 3A). C21 was observed to induce eosinophilic leukocytosis. Peripheral blood eosinophilia in C21 treated mice rose significantly from 2%-12% by 6 hours post injection, compared to no significant change in eosinophil levels in the fucoidan treated mice (Figure 3B). At this dose of C21 the resulting 12% eosinophilia showed a significant increase from the 9% observed at low dose brief (50μg/mouse) in previous experiments. Both C21 and fucoidan induced neutrophilic leukocytosis, indicative of haematopoietic progenitor and stem cell release. Neutrophil levels were elevated from 20% to 47% by 6 hours post injection in the peripheral blood of C21 treated mice, compared to a 20%- 82% rise in fucoidan treated mice (Figure 3C).

Example 6: Induction of leukocytosis and SDF-1α release into the peripheral blood by synthetically–made glycan mimetics (SSGMs)

A larger panel of SSGMs (1mg/mouse or 50mg/kg) were tested for their ability to induce eosinophilic and/or neutrophilic leukocytosis, and stromal derived factor-1 alpha (SDF-1α) release into the peripheral blood. Blood smears from the tail vein were taken 0, 2 and 6 hours after intravenous injection, and the percentage of neutrophils and eosinophils were determined in peripheral blood. C21, C10 and C22 were found to induce eosinophil leukocytosis (Figure 4A, Figures 4C-4E, Figure 5), while many of the SSGMs tested induced neutrophilic leukocytosis (Figure 4B, Figures 4C-4E, Figure 5). These changes were accompanied by a corresponding decrease in the percentage of lymphocytes in both groups over the 6 hour experiment (Figures 4C-4E).

Stromal cell-derived factor 1 alpha (SDF-1α) levels in plasma taken from blood collected 0, and 6 hours post SSGM challenge were determined using an R&D Duo assay (DY460) (Minneapolis, MN). Significant SDF-1α release into the peripheral blood in response to SSGM treatment was apparent at 6 hours post-injection, corresponding to the observed neutrophilia (Figure 5). SDF-1α release is an important factor used as an indicator of bone marrow progenitor/stem cell release into the peripheral blood. Due to the complex nature of fucoidan (a large polysaccharide) it was not surprising to see such a significant increase in SDF-1α release.

Example 7: Trafficking of enhanced green fluorescent protein positive ($EGFP^+$) cells in response to challenge with SSGMs

Two million splenocytes from $EGFP^+$ transgenic mice (SPF facility, John Curtin School of Medical Research) were administered intravenously to mice, followed by a second intravenous injection of 100 μL of C21 (10mg/mL), fucoidan (10mg/mL), sulfated maltopentasaccharide (10mg/mL) or saline. Blood and spleen samples were taken and processed 30 minutes post-injection, then analysed for $EGFP^+$ cells by FACS. Within 30 minutes, C21 and MPS treatment induced a general leukocytosis (Figure 6A) and neutrophilic leukocytosis (Figure 6B). Unlike fucoidan, neither C21 or MPS induced a decrease in trafficking of adoptively transferred EGFP+ splenocytes. This was indicated by the retention of labelled cells in the peripheral blood (Figure 6C) and a reduction in the trafficking of labelled cells to the spleen (Figure 6D) after fucoidan treatment.

Example 8: Optimal biosensor analysis of the effect of sulfated oligosaccharides on the association between heparin and eotaxin

Competition binding studies were performed on a BIAcore 2000 surface plasmon resonance-based biosensor (BIAcore, Uppsala, Sweden) using biotinylated bovine lung heparin immobilized on a CM5 sensor chip (BIAcore). Eotaxin (50nM) was co-incubated with each SSGM and injected into the flow-cells of the biosensor with binding monitored for 7.9 mins for each solution of eotaxin and SSGM. The level of eotaxin binding to the immobilised heparin in the absence of any sulfated oligosaccharides was set as 100% binding. The level of eotaxin binding relative to this amount was measured in the presence of each SSGM. These studies showed that 1 μM C21 inhibited the heparin/eotaxin association by 43% (Figure 7A). No other SSGM tested inhibited heparin/eotaxin binding by more than 10%.

Biosensor analysis of a large panel of SSGMs confirmed the ability of large molecular weight heparins, mast cell produced GAGs, but not heparan sulfate to competitively inhibit eotaxin binding to heparin, with IC50's below 20nM (Figure 7B). Interestingly the other mast cell associated GAG chondroitin sulfate E, although less effective than LMW heparins, induced a moderate inhibition with an IC50 at 130nM. Of particular interest was the ability of C21 to induce strong inhibition of eotaxin/heparin binding.

Example 9: Haematopoietic progenitor cell and stem cell mobilisation following treatment with C21, maltopentaose sulfate (MPS) and fucoidan.

C57BL/6 mice which strongly express stem cell antigen-1 (Sca) were injected on Day 0 with cyclophosphamide and blood samples were taken on days 0, 2, 4, 6 and 8. Six hours prior to sacrifice, mice were intravenously injected with 50mg/kg of the appropriate SSGM, or not injected and left as controls.

FACS analysis was used to analyse the release of HPC (committed progenitor cells) (Figure 8A). HPC were identified during FACS analyses as lineage negative (Lin^-), stem cell factor receptor positive ($c\text{-}kit^+$) cells, where Lin^- cells were characterized as $CD3^-$, $B220^-$, $CD11b^-$. The effect of cyclophosphamide +/- SSGM treatment on Lin^-c-Kit+ cell numbers in the peripheral blood was evaluated by FACS in groups treated with cyclophosphamide (Cyc) alone, Cyc+Saline, Cyc+Fucoidan, Cyc+MPS, Cyc+C21 and untreated mice. Quantitative analysis confirmed that C21 co-treatment with Cyc induced the highest increase in Lin-Kit+ (progenitor) cell release (Figure 8B). Unlike fucoidan,

C21 induced a significant increase in Lin-Kit+ cell release compared to Cyc treatment alone.

HSC were identified during FACS analyses as lineage negative (Lin^-) (where Lin^- cells are further defined as $CD3^-$, $B220^-$, $CD11b^-$), stem cell factor receptor positive (c-kit^+), stem cell antigen-1 positive (Sca^+) cells. The effect of cyclophosphamide (Cyc) +/- SSGM treatment on $Lin^-c\text{-}Kit^+Sca^+$ cell numbers in the peripheral blood was evaluated by FACS in groups treated with cyclophosphamide (Cyc) alone (Figure 9A), Cyc+Saline, Cyc+Fucoidan, Cyc+MPS, Cyc+C21 and untreated mice. Quantitative analysis confirmed that C21 co-treatment with Cyc induced the highest increase in Lin-Kit+Sca+ (stem) cell release, shown as a percentage of cells in total peripheral blood (Figure 9B). Unlike fucoidan, C21 induced a significant increase in Lin-Kit+Sca+ (stem) cell release compared to Cyc treatment alone. In addition, quantitative analysis confirmed that C21 co-treatment with Cyc induced the highest increase in Lin-Kit+Sca+ (stem) cell release, shown as a percentage of Lin-Kit+ cells in peripheral blood (Figure 9C). Unlike fucoidan, C21 induced a significant increase in the proportion of Sca+ cells in the Lin-Kit+ cell population, when compared to Cyc treatment alone (Figure 9C).

Example 10 – Compositions

A pharmaceutical composition of the present invention for intramuscular injection could be prepared to contain 1-5 mL sterile buffered water, and 50 mg of low molecular weight anionic glycan mimetic.

Similarly, a pharmaceutical composition for intravenous infusion may comprise 250 mL of sterile Ringer's solution, and 25 mg of a low molecular weight anionic glycan mimetic.

Capsule Composition

A pharmaceutical composition of low molecular weight anionic glycan mimetic in the form of a capsule may be prepared by filling a standard two-piece hard gelatin capsule with 50 mg of a low molecular weight glycosaminoglycan mimetic, in powdered form, 100 mg of lactose, 35 mg of talc and 10 mg of magnesium stearate.

Injectable Parenteral Composition

A pharmaceutical composition of this invention in a form suitable for administration by injection may be prepared by mixing 25 mg by weight of low molecular

weight anionic glycan mimetic in 10% by volume propylene glycol and water. The solution is sterilised by filtration.

Composition for Inhalation Administration

For an aerosol container with a capacity of 20-30 mL: a mixture of 25 mg of a low molecular weight anionic glycan mimetic with 0.5-0.8% by weight of a lubricating agent, such as polysorbate 85 or oleic acid, is dispersed in a propellant, such as freon, and put into an appropriate aerosol container for either intranasal or oral inhalation administration.

CLAIMS

1. A method for mobilisation of cells capable of circulating around the body of a subject, said method comprising administration to the subject of an effective amount of low molecular weight anionic glycan mimetic, or a pharmaceutically acceptable salt thereof.

2. The method of claim 1 wherein said cells are stem cells.

3. The method of claim 2 wherein said stem cells are selected from the group consisting of hematopoietic stem cells, mesenchymal stem cells, bone and osteoclast stem cells, hepatic and hepatic endothelial stem cells, myogenic stem cells, endothelial stem cells, epithelial stem cells, leukocytes, platelets and erythrocytes.

4. The method of claim 3 wherein said hematopoietic stem cells are selected from the group consisting of lymphoid progenitor cells, myeloid progenitor cells, platelet progenitor cells and erythroid progenitor cells.

5. The method of claim 4 wherein said lymphoid progenitor cells are selected from the group consisting of B cell progenitor cells, T cell progenitor cells and dendritic cell progenitor cells.

6. The method of claim 4 wherein said myeloid progenitor cells are selected from the group consisting of basophilic progenitor cells, eosinophilic progenitor cells, neutrophilic progenitor cells, monocytic progenitor cells, mast cell progenitor cells, macrophage progenitor cells and dendritic cell progenitor cells.

7. The method of claim 4 wherein said platelet progenitor cells are megakaryocytes.

8. The method of claim 4 wherein said erythroid progenitor cells are erythroblasts.

9. The method of any one of claims 1-8, wherein said low molecular weight anionic glycan mimetic is selected from the group consisting of low molecular weight heparan sulfate mimetics, low molecular weight glycosaminoglycan mimetics, monosaccharides, disaccharides, oligosaccharides, cyclic oligosaccharides, cyclitols, arylene ureas, pseudo sugars, and mixtures thereof, each of which may possess one or more anionic residues.

10. The method of claim 9 wherein said cyclic oligosaccharides are cyclodextrins.

11. The method of any one of claims 1-9 wherein said low molecular weight anionic glycan mimetic comprises one or more sulfate, phosphate or carboxylate groups.

12. A method for mobilising mature cells in a subject, said method comprising administration to the subject of an effective amount of a low molecular weight anionic glycan mimetic, or a pharmaceutically acceptable salt thereof.

13. The method of claim 12 wherein said mature cells are selected from the group consisting of lymphocytes, leukocytes, platelets and erythrocytes.

14. The method of claim 13 wherein said lymphocytes are selected from the group consisting of T cells, natural killer cells, B cells and natural killer T cells.

15. The method of claim 13 wherein said leukocytes are selected from the group consisting of neutrophils, basophils, monocytes, eosinophils, mast cells, dendritic cells, megakaryocytes and macrophages.

16. A method for mobilising stem cells in a subject, said method comprising administration to the subject of an effective amount of a low molecular weight anionic glycan mimetic or a pharmaceutically acceptable salt thereof.

17. A method for the treatment of a condition in a subject where a stem cell transplant is required, said method comprising administration to the subject of a therapeutically effective amount of a low molecular weight anionic glycan mimetic, or a pharmaceutically acceptable salt thereof.

18. The method of claim 17 wherein said condition is a haematological disease, an immunologic disease or a neoplastic disease.

19. The method of claim 17 or 18 wherein said condition is selected from the group consisting of leukemia and lymphoma, acute lymphoblastic leukemia, acute myoblastic leukemia, chronic myelogenous leukemia, Hodgkin's disease, multiple myeloma, non-Hodgkin's lymphoma, childhood brain tumors, neuroblastoma, inherited blood disorders, inherited inborn errors of metabolism that are treated with bone marrow transplants, haemopoietic stem cell rescue in cancer therapy, Graft-versus-host treatment of cancer, systemic lupus erythematosus, cardiovascular diseases, muscle degenerative diseases requiring myogenic regeneration, and nervous system disorders requiring re-generation of neural tissue, re-myelination of nerves and glial cell repopulation.

20. The method of claim 19 wherein said inherited blood disorders are selected from the group consisting of aplastic anaemia, beta-thalassemia, globoid cell leukodystrophy, X-linked lymphoproliferative syndrome and severe combined immunodeficiency syndrome.

21. The method of claim 19 wherein said inherited inborn errors of metabolism that are treated with bone marrow transplants are selected from the group consisting of Hunter's syndrome, Hurler's syndrome, fucosidosis, and Lesch Nyhan syndrome.

22. The method of claim 19 wherein said cardiovascular diseases require myocardial regeneration using mesenchymal stem cell transplantation.

23. The method of claim 19 wherein said muscle degenerative disease is muscular dystrophy.

24. The method of claim 19 wherein said nervous system disorders requiring re-generation of neural tissue, re-myelination of nerves and glial cell repopulation are selected from the group consisting of Parkinson's disease, multiple sclerosis, Alzheimer's disease and motor neuron disease.

25. The method of claim 17 wherein said stem cells are pluripotent or are haematopoietic stem cells.

26. The method of claim 25 wherein said haematopoietic stem cells are selected from the group consisting of lymphoid progenitor cells, myeloid progenitor cells, platelet progenitor cells, and erythroid progenitor cells.

27. The method of claim 26 wherein said lymphoid progenitor cells are selected from the group consisting of B cell progenitor cells, T cell progenitor cells and dendritic cell progenitor cells.

28. The method of claim 26 wherein said myeloid progenitor cells are selected from the group consisting of basophilic progenitor cells, eosinophilic progenitor cells, neutrophilic progenitor cells, monocytic progenitor cells, mast cell progenitor cells, macrophage progenitor cells, and dendritic cell progenitor cells.

29. The method of claim 26 wherein said platelet progenitor cells are megakaryocytes.

30. The method of claim 26 wherein said erythroid progenitor cells are erythroblasts.

31. The method of claim 17 wherein said stem cell is a megakaryocyte.

32. The method of claim 17 wherein said condition is selected from the group consisting of drug-induced thrombocytopenia, idiopathic thrombocytopenia purpura, Glanzmann thrombasthenia, Bernard-Soulier syndrome, Platelet-type von Willebrand disease, Hermansky-Pudlak and Chediak-Higashi syndromes, and defective platelet procoagulant activity (Scott syndrome).

33. A method of claim 32 wherein said drug-induced thrombocytopenia is induced by heparin.

34. A composition for mobilisation of cells capable of circulating around the body of a subject, said composition comprising a low molecular weight anionic glycan

35. The composition of claim 34 wherein said low molecular weight anionic glycan mimetic or pharmaceutically acceptable salt thereof, and agent, are synergistic in terms of their ability to mobilise cells capable of circulating around the body of the subject.

36. The composition of claim 34 or 35 wherein the agent may be selected from the group consisting of G-CSF, GM-CSF, IL-8, AMD-3100, chemotherapeutic drugs, and chemotherapeutic agents.

37. The composition of claim 36 wherein said chemotherapeutic drugs are selected from the group consisting of methotrextrate, docetaxel, paclitaxel, epirubicin, mitoguazone, amifostine, adriamycin, taxol, fluorouracil, melphalan, cisplatin, alpha interferon, COMP (cyclophosphamide, vincristine, methotrexate and prednisone), etoposide, mBACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine and dexamethasone), PROMACE/MOPP (prednisone, methotrexate (w/leucovin rescue), doxorubicin, cyclophosphamide, taxol, etoposide/mechlorethamine, vincristine, prednisone and procarbazine), vincristine, vinblastine, angioinhibins, TNP-470, pentosan polysulfate, platelet factor 4, angiostatin, LM-609, SU-101, CM-101, Techgalan, thalidomide and SP-PG.

38. The composition of claim 36 wherein said chemotherapeutic agents are selected from the group consisting of alkylating agents, nitrosoureas, alkyl sulfonates, triazines, ethylenimines, folic acid analogues, pyrimidine analogues, purine analogues, antitumour antibiotics, the anthracyclines, hormones and hormone antagonists, cisplatin and brequinar.

39. The composition of claim 38 wherein said alkylating agents are nitrogen mustards.

40. The composition of claim 39 wherein said nitrogen mustards are selected from the group consisting of mechlorethamine, melphan, chlorambucil, cyclophosphamide and ifosfamide.

41. The composition of claim 38 wherein said nitrosoureas are selected from the group consisting of carmustine, lomustine, semustine and streptozocin.

42. The composition of claim 38 wherein said alkyl sulfonate is busulfan.

43. The composition of claim 38 wherein said triazine is dacarbazine.

44. The composition of claim 38 wherein said ethylenimines are thiotepa and hexamethylmelamine.

45. The composition of claim 38 wherein said folic acid analogue is methotrexate.

46. The composition of claim 38 wherein said pyrimidine analogues are 5-fluorouracil and cytosine arabinoside.

47. The composition of claim 38 wherein said purine analogues are 6-mercaptopurine and 6-thioguanine.

48. The composition of claim 38 wherein said antitumour antibiotic is actinomycin D.

49. The composition of claim 38 wherein said anthracyclines are selected from the group consisting of doxorubicin, bleomycin, mitomycin C and mithramycin.

50. The composition of claim 38 wherein said hormones and hormone antagonists are tamoxifen and corticosteroids.

51. The method of claim 1 or claim 12 wherein said mobilisation further comprises entry of one or more of said cells into a tissue.

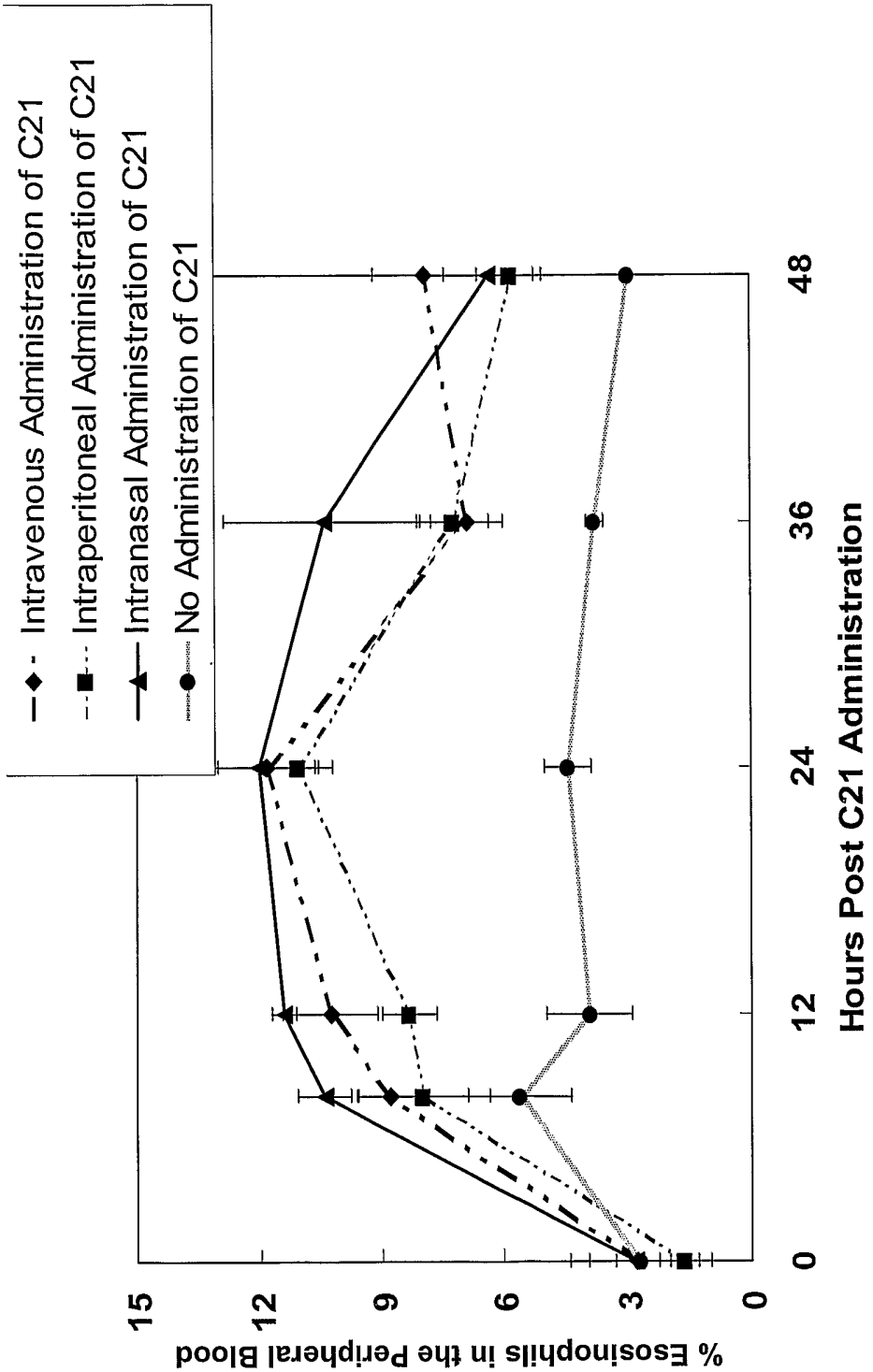

Intravenous Administration of C21
Intraperitoneal Administration of C21
Intranasal Administration of C21
No Administration of C21
% Eosinophils in the Peripheral Blood
0
3
6
9
12
15
0
12
24
36
48
Hours Post C21 Administration

Figure 1

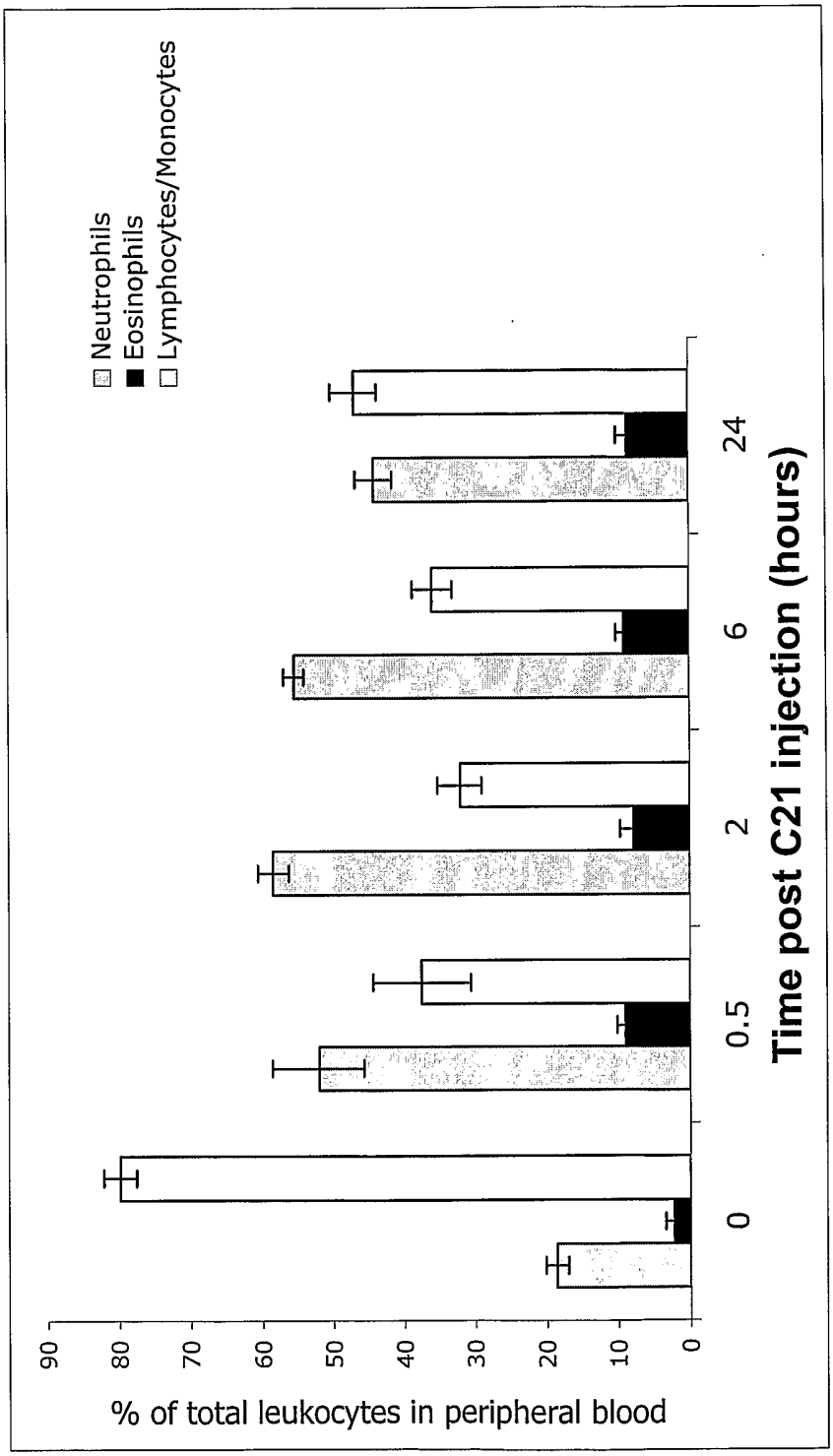

Neutrophils
Eosinophils
Lymphocytes/Monocytes
% of total leukocytes in peripheral blood
0
10
20
30
40
50
60
70
80
90
0
0.5
2
6
24
Time post C21 injection (hours)

Figure 2A

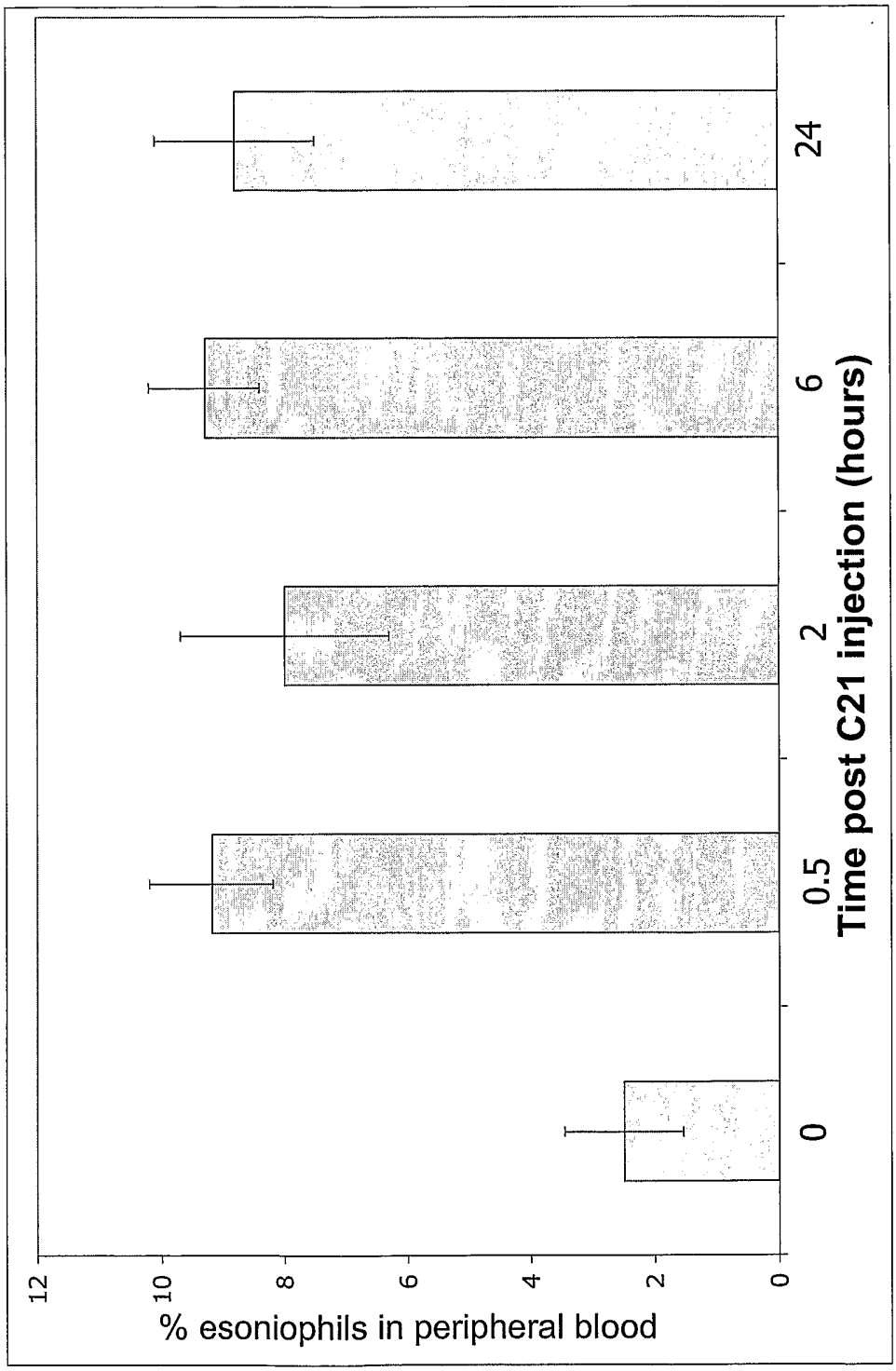

% esoniophils in peripheral blood
0
2
4
6
8
10
12
Time post C21 injection (hours)
0
0.5
2
6
24

Figure 2B

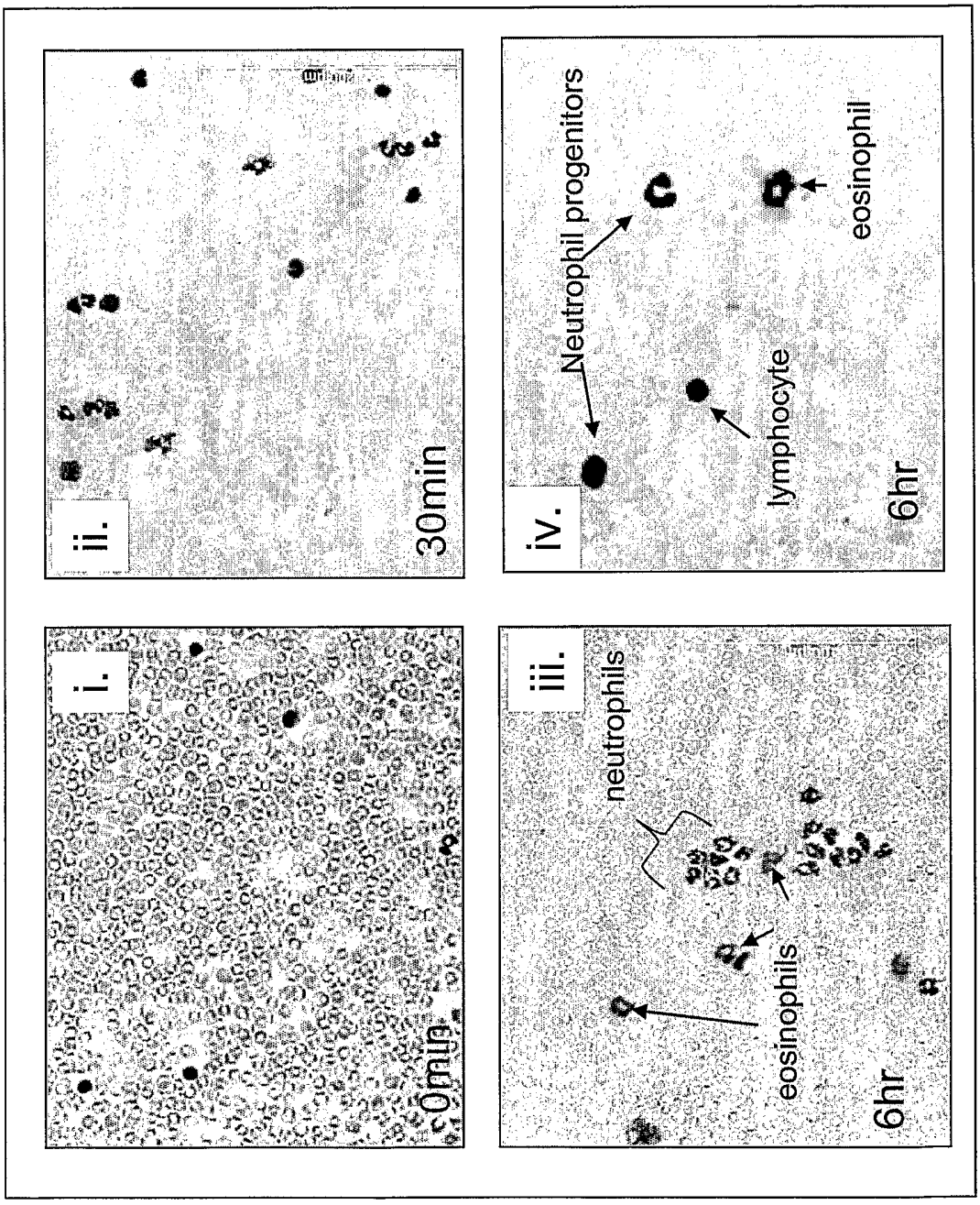

i.
0min
ii.
30min
iii.
neutrophils
eosinophils
6hr
iv.
Neutrophil progenitors
lymphocyte
eosinophil
6hr

Figure 2C

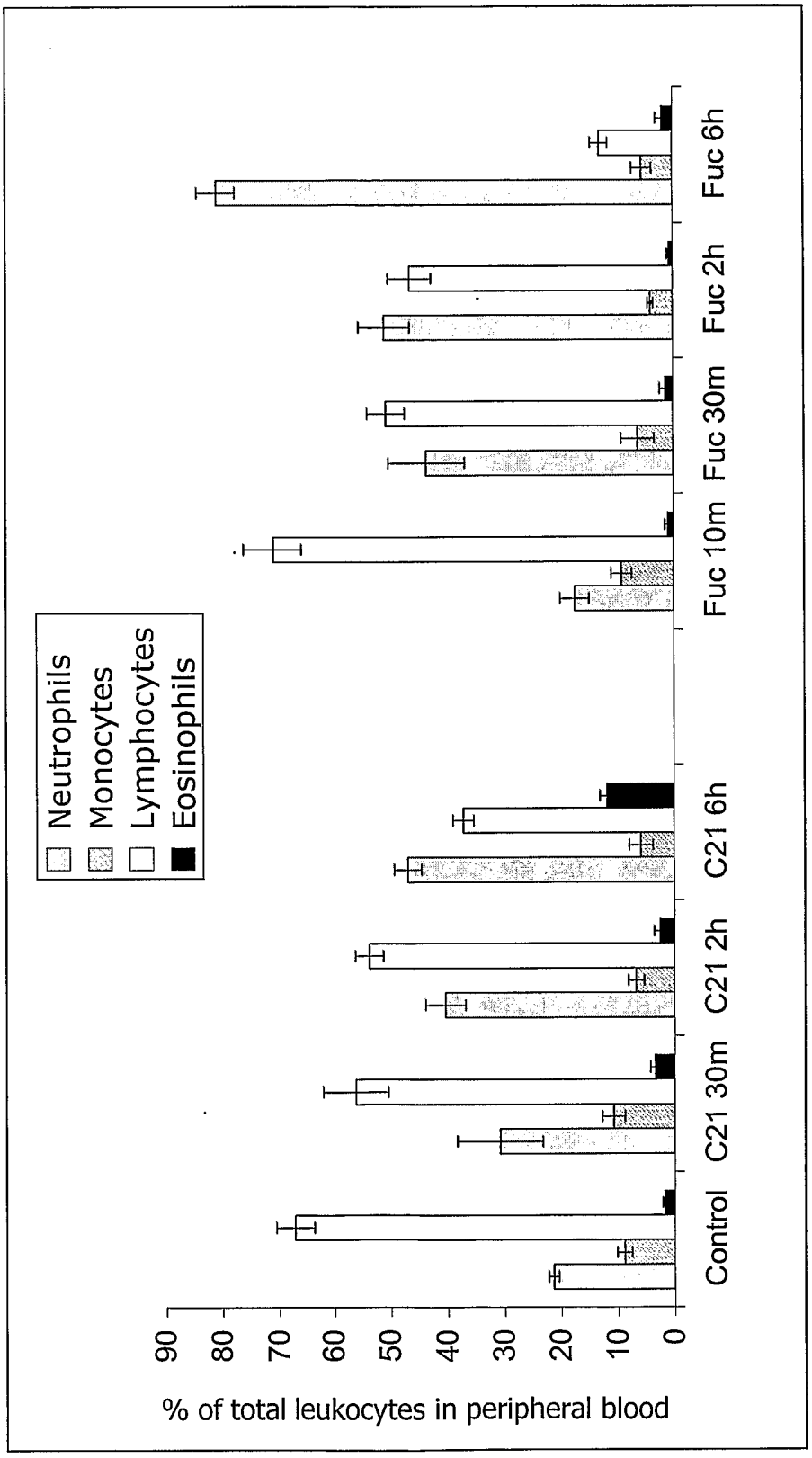

Neutrophils
Monocytes
Lymphocytes
Eosinophils
Control
C21 30m
C21 2h
C21 6h
Fuc 10m
Fuc 30m
Fuc 2h
Fuc 6h
90
80
70
60
50
40
30
20
10
0
% of total leukocytes in peripheral blood

Figure 3A

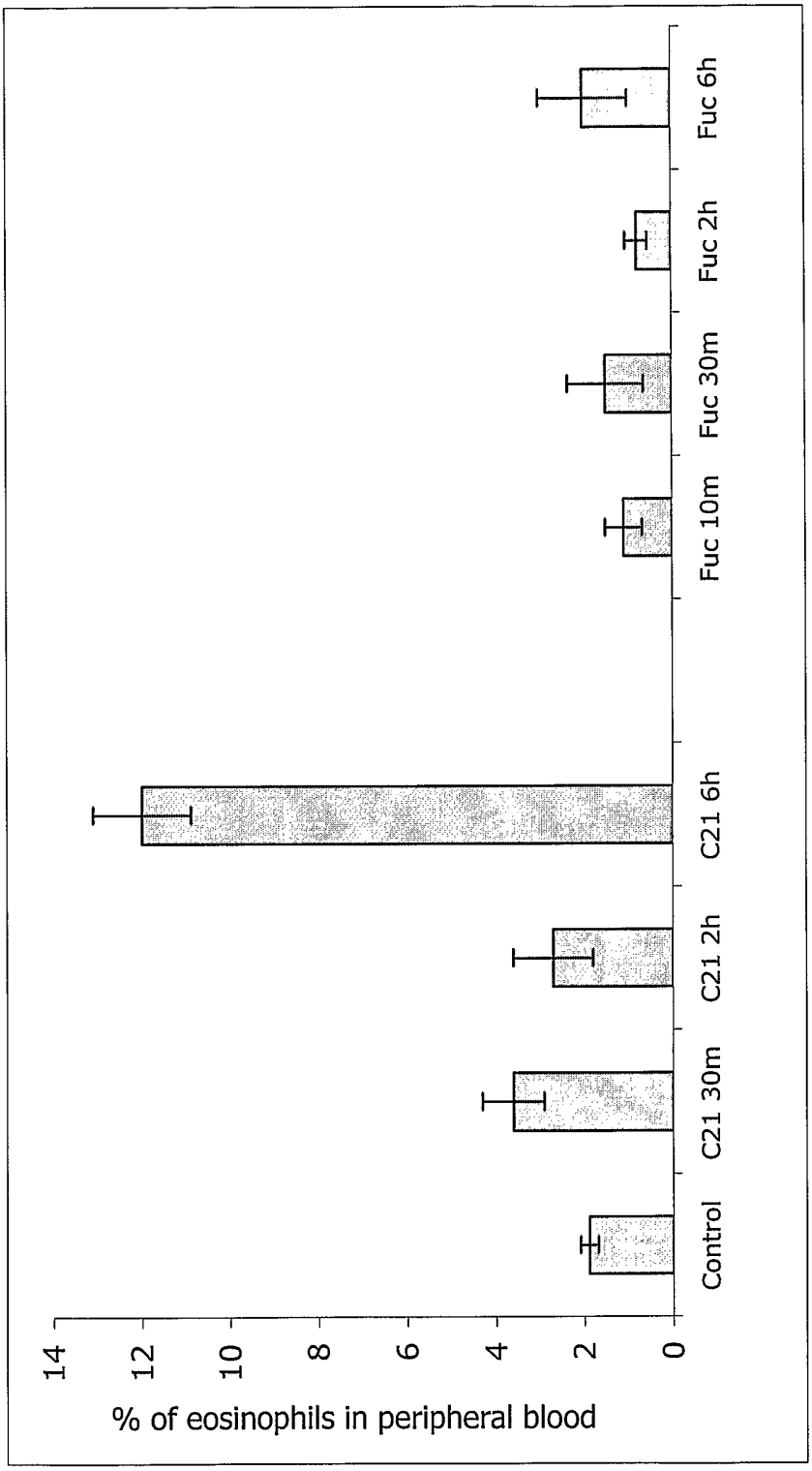

% of eosinophils in peripheral blood
0
2
4
6
8
10
12
14
Control
C21 30m
C21 2h
C21 6h
Fuc 10m
Fuc 30m
Fuc 2h
Fuc 6h

Figure 3B

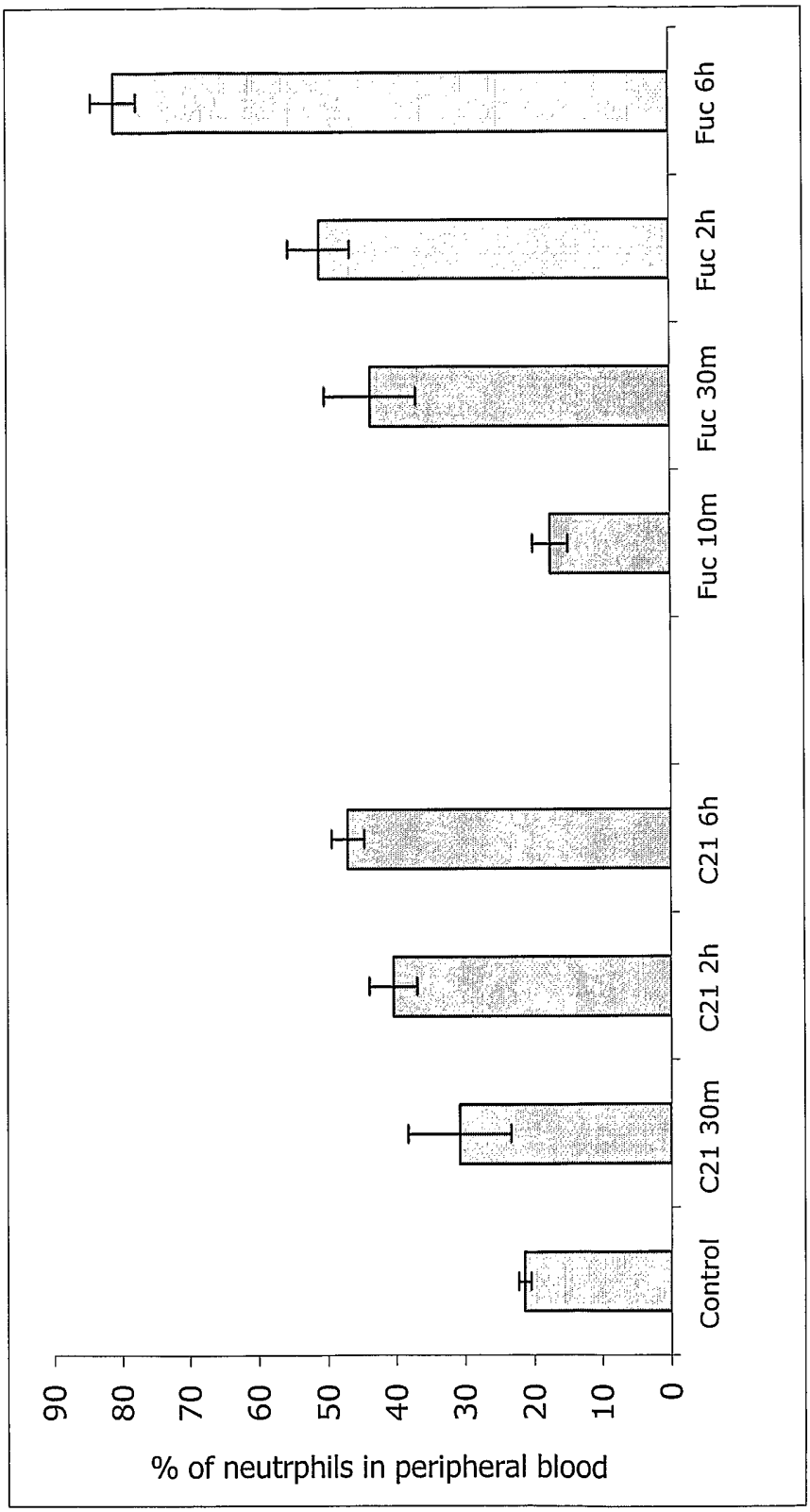

Control
C21 30m
C21 2h
C21 6h
Fuc 10m
Fuc 30m
Fuc 2h
Fuc 6h
0
10
20
30
40
50
60
70
80
90
% of neutrphils in peripheral blood

Figure 3C

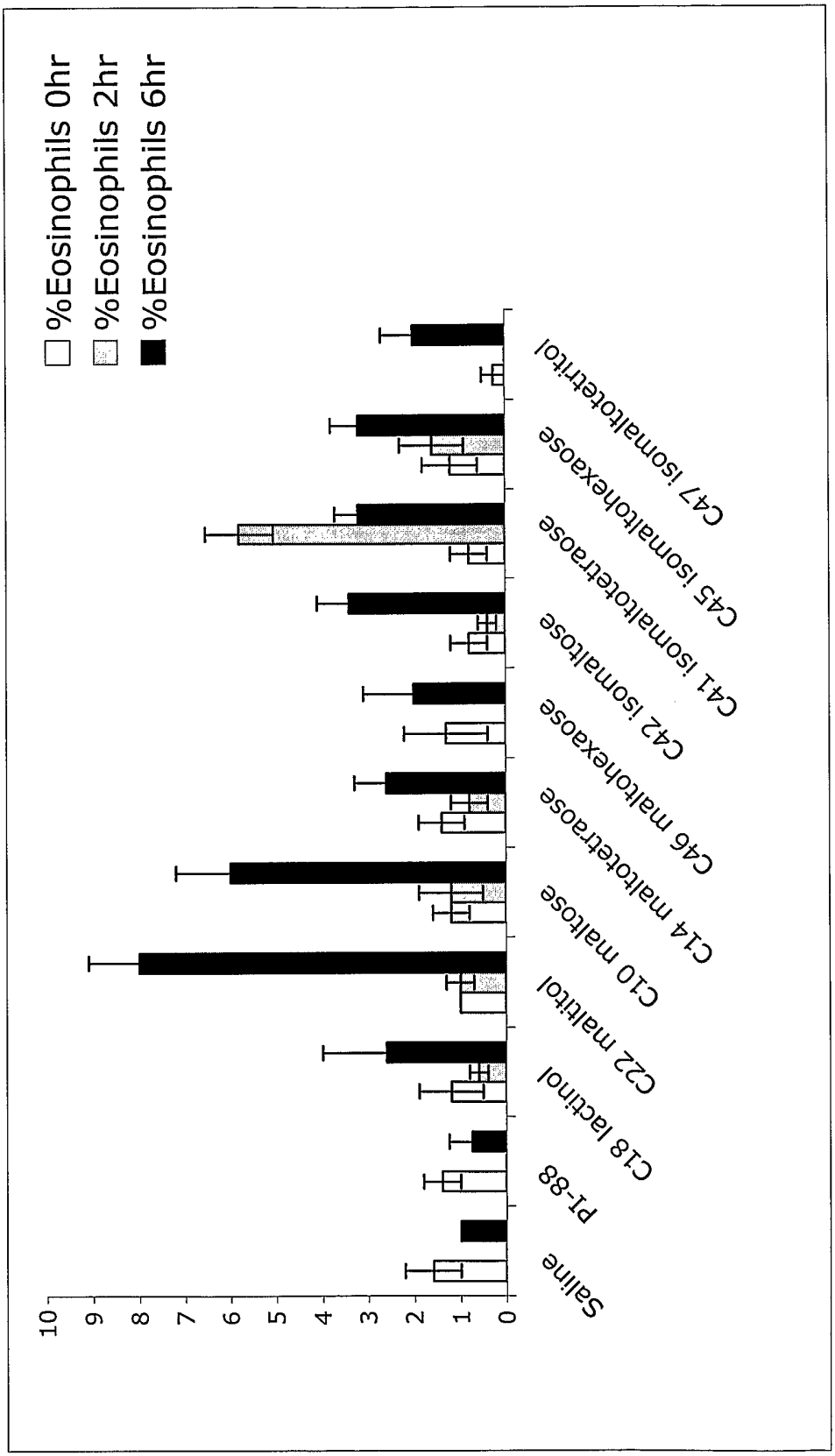

%Eosinophils 0hr
%Eosinophils 2hr
%Eosinophils 6hr
10
9
8
7
6
5
4
3
2
1
0
Saline
PI-88
C18 lactinol
C22 maltitol
C10 maltose
C14 maltotetraose
C46 maltohexaose
C42 isomaltose
C41 isomaltotetraose
C45 isomaltohexaose
C47 isomaltotetritol

Figure 4A

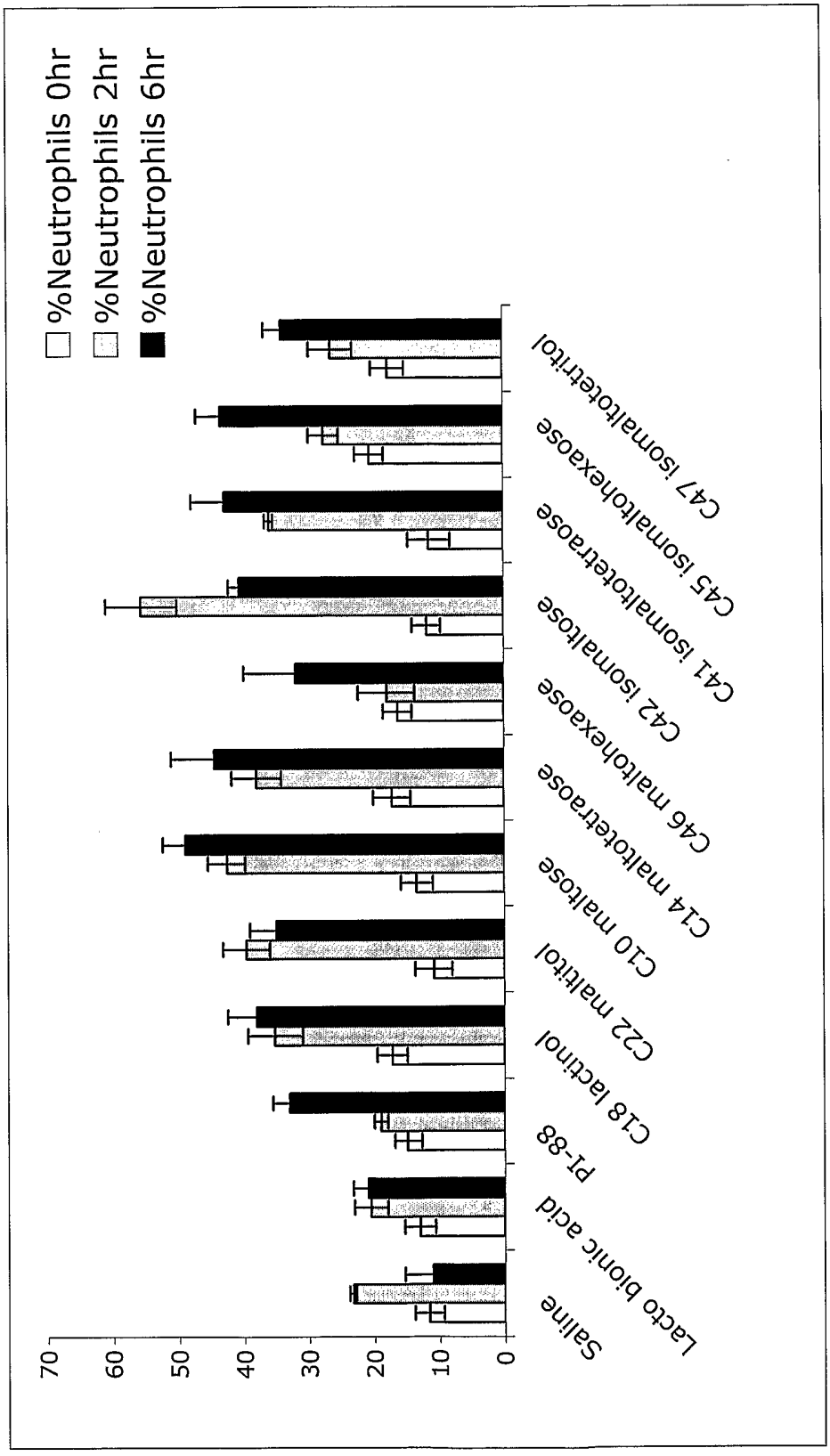

%Neutrophils 0hr
%Neutrophils 2hr
%Neutrophils 6hr
0
10
20
30
40
50
60
70
Saline
Lacto bionic acid
PI-88
C18 lactinol
C22 maltitol
C10 maltose
C14 maltotetraose
C46 maltohexaose
C42 isomaltose
C41 isomaltotetraose
C45 isomaltohexaose
C47 isomaltotetritol

Figure 4B

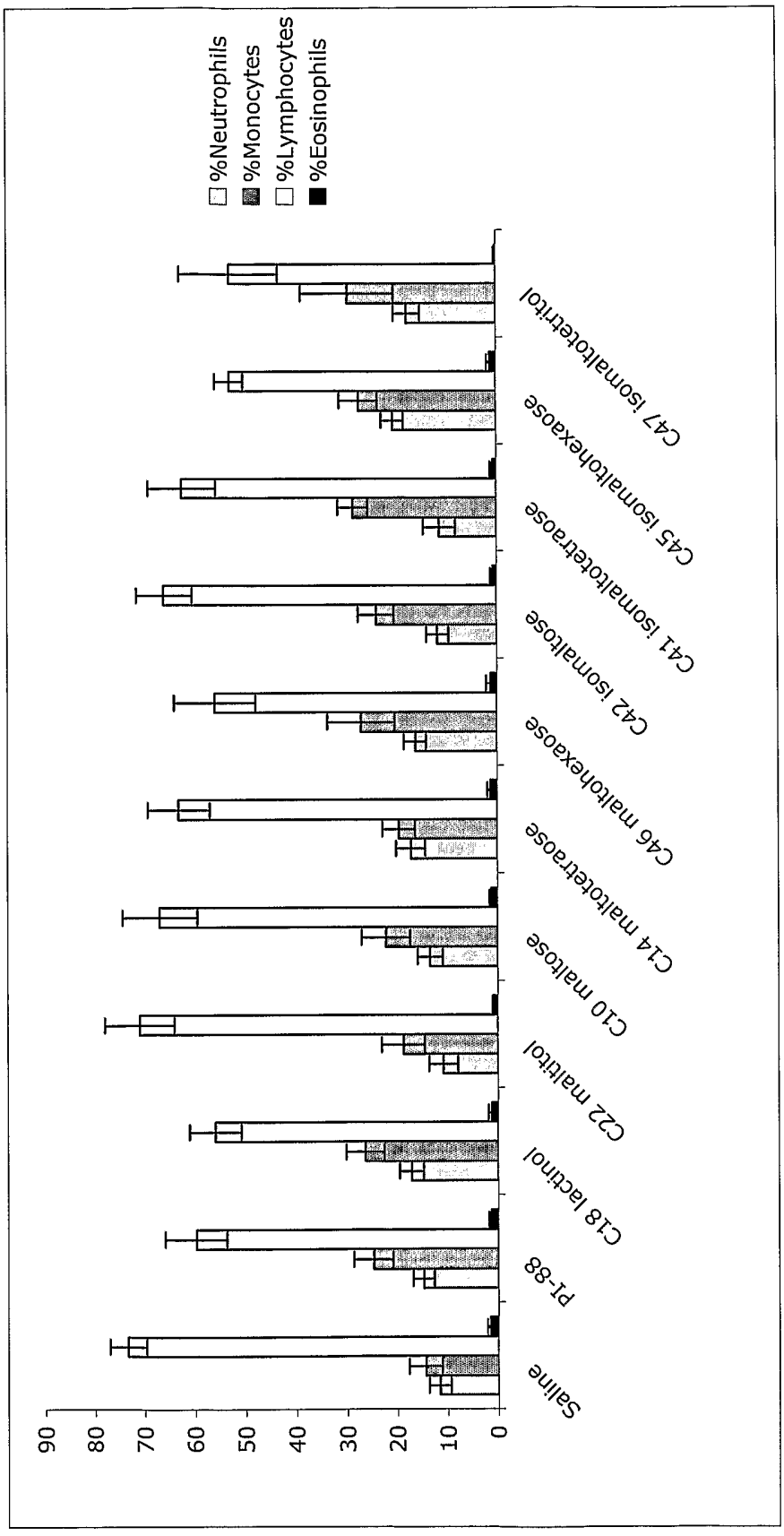

%Neutrophils
%Monocytes
%Lymphocytes
%Eosinophils
Saline
PI-88
C18 lactinol
C22 maltitol
C10 maltose
C14 maltotetraose
C46 maltohexaose
C42 isomaltose
C41 isomaltotetraose
C45 isomaltohexaose
C47 isomaltotetritol
0
10
20
30
40
50
60
70
80
90

Figure 4C

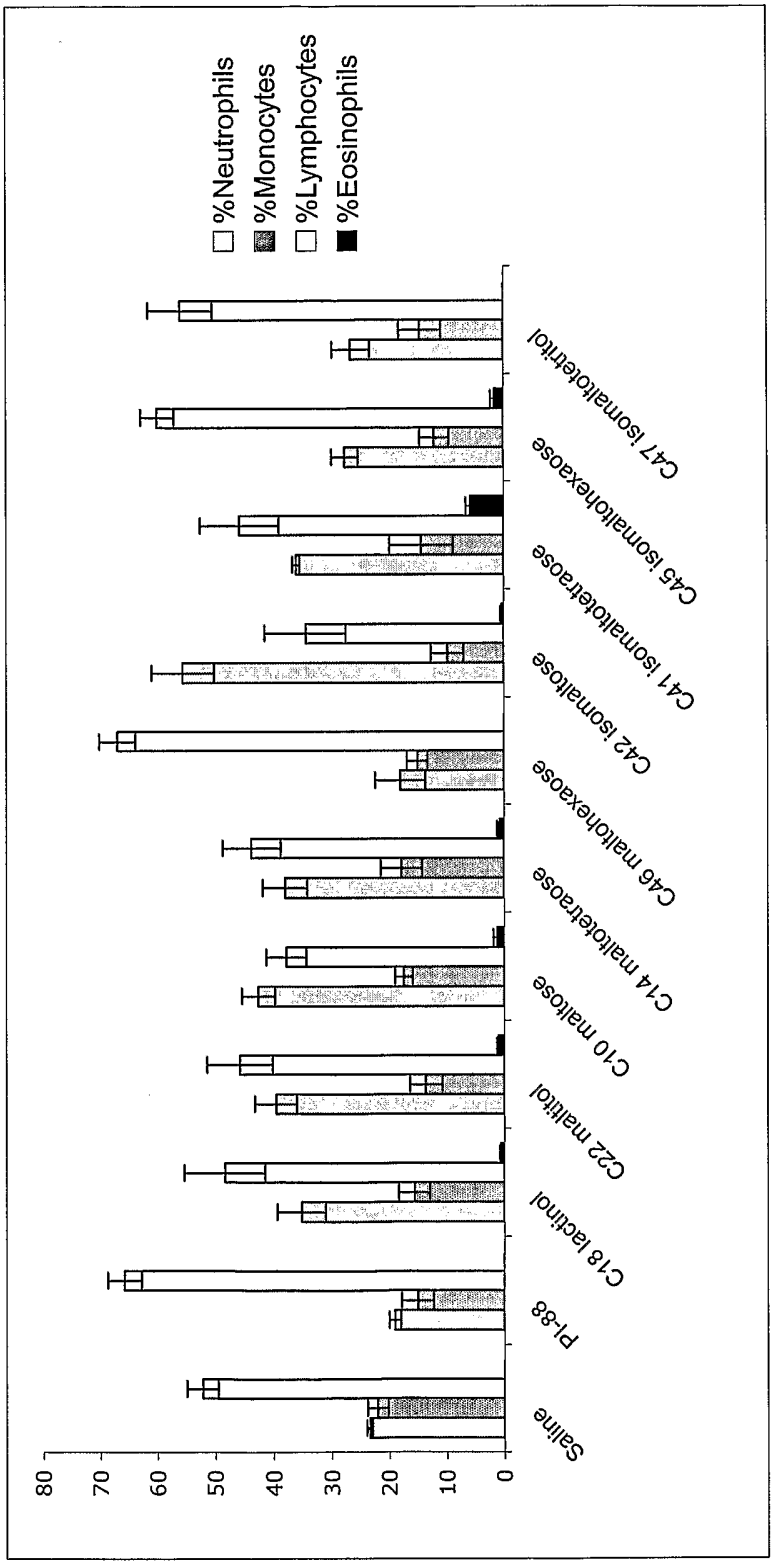

%Neutrophils
%Monocytes
%Lymphocytes
%Eosinophils
Saline
Pl-88
C18 lactinol
C22 maltitol
C10 maltose
C14 maltotetraose
C46 maltohexaose
C42 isomaltose
C41 isomaltotetraose
C45 isomaltohexaose
C47 isomaltoheptitol
0
10
20
30
40
50
60
70
80

Figure 4D

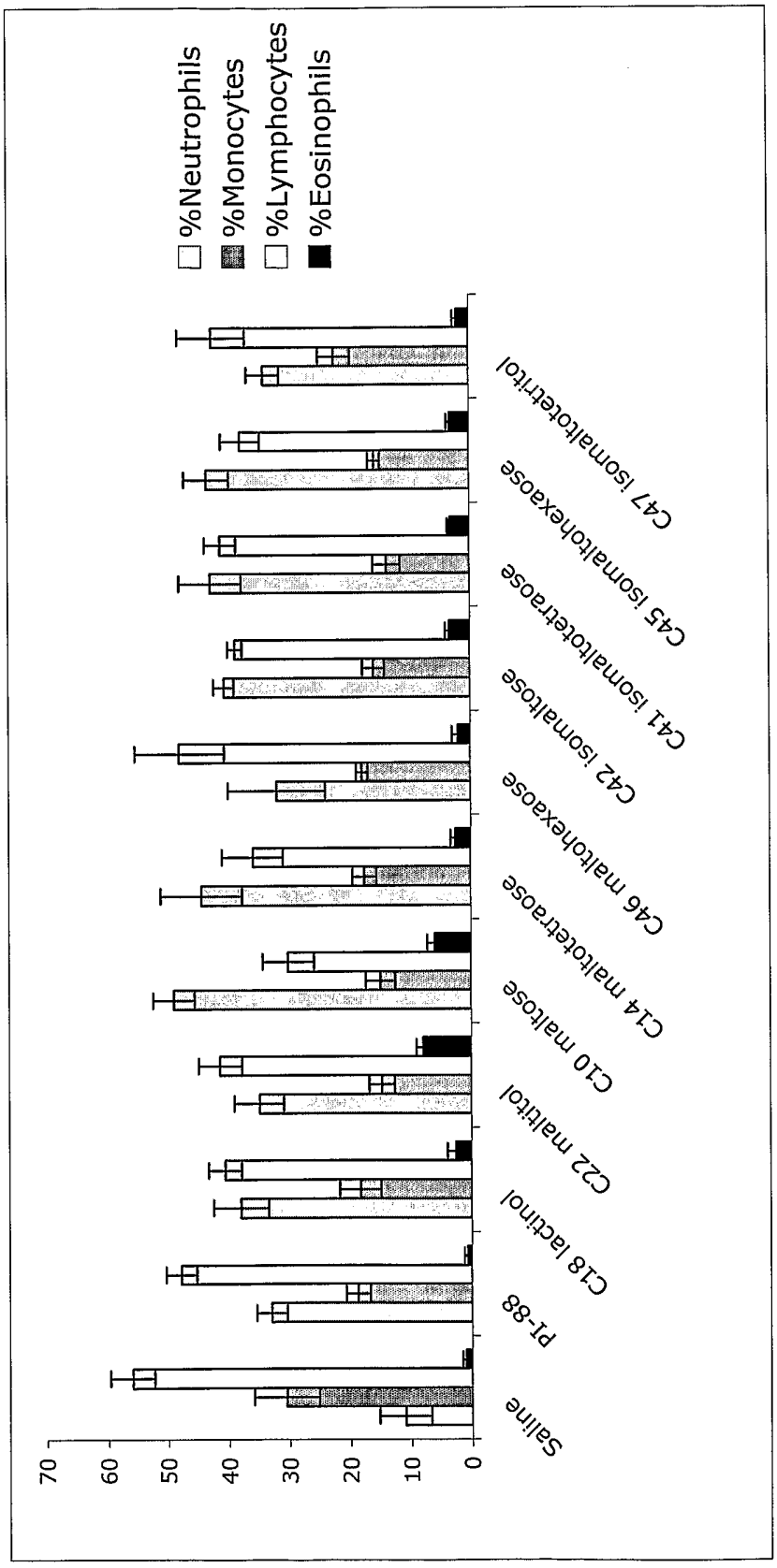

%Neutrophils
%Monocytes
%Lymphocytes
%Eosinophils
70
60
50
40
30
20
10
0
Saline
PI-88
C18 lactitol
C22 maltitol
C10 maltose
C14 maltotetraose
C46 maltohexaose
C42 isomaltose
C41 isomaltotetraose
C45 isomaltohexaose
C47 isomaltotetritol

Figure 4E

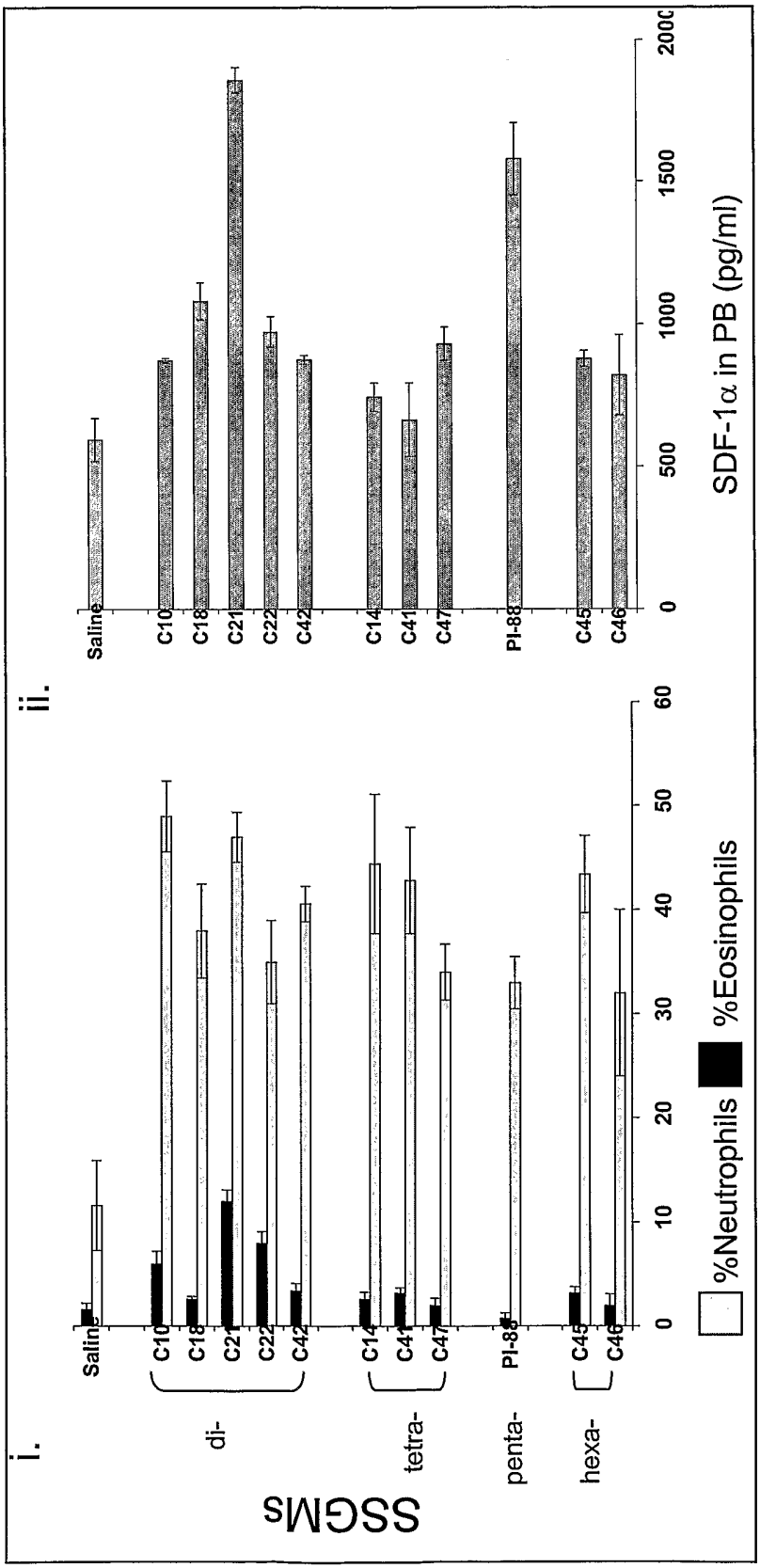

i.
ii.
SSGMs
di-
tetra-
penta-
hexa-
Saline
C10
C18
C21
C22
C42
C14
C41
C47
PI-88
C45
C46
%Neutrophils
%Eosinophils
SDF-1α in PB (pg/ml)
0
10
20
30
40
50
60
500
1000
1500
2000

Figure 5

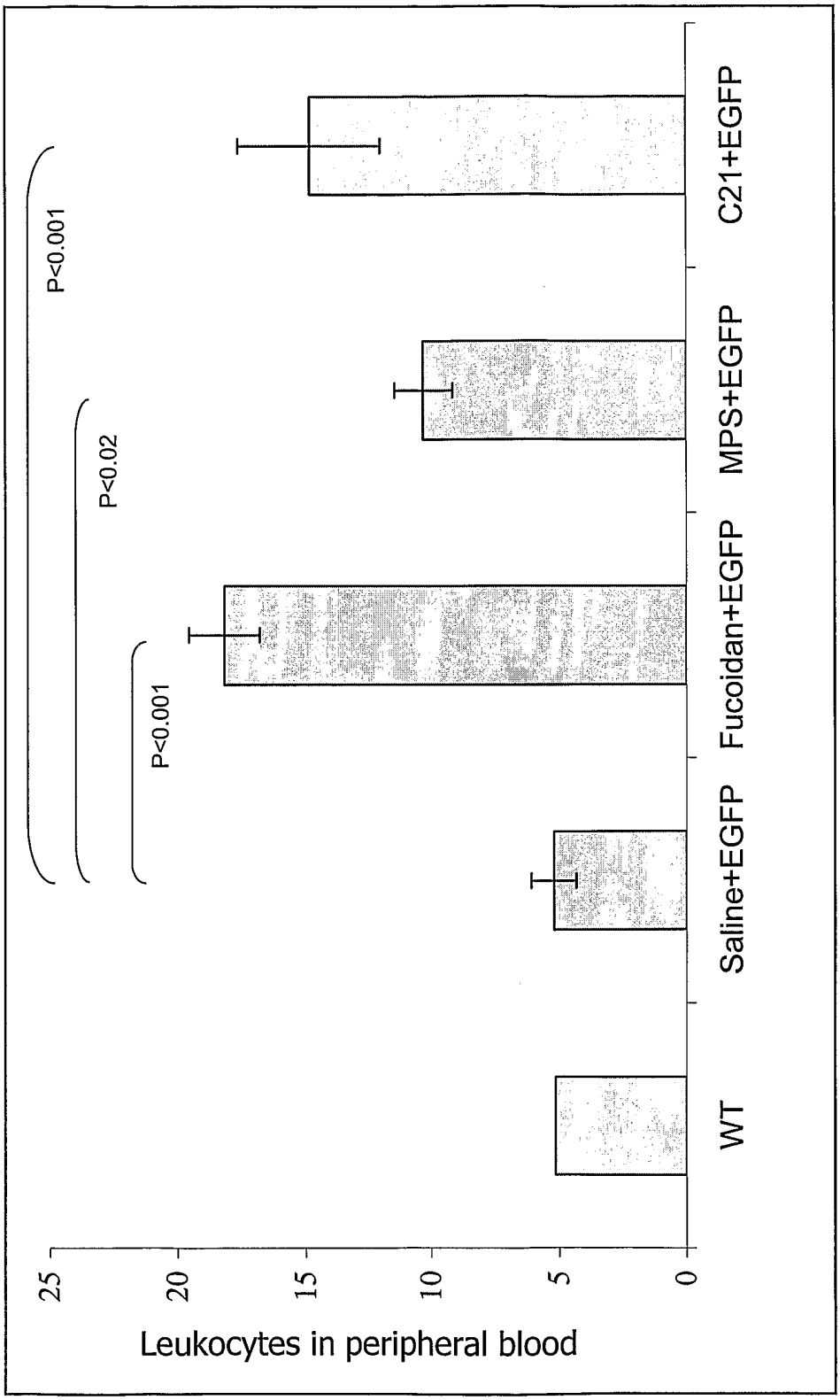

Leukocytes in peripheral blood
25
20
15
10
5
0
WT
Saline+EGFP
Fucoidan+EGFP
MPS+EGFP
C21+EGFP
P<0.001
P<0.02
P<0.001

Figure 6A

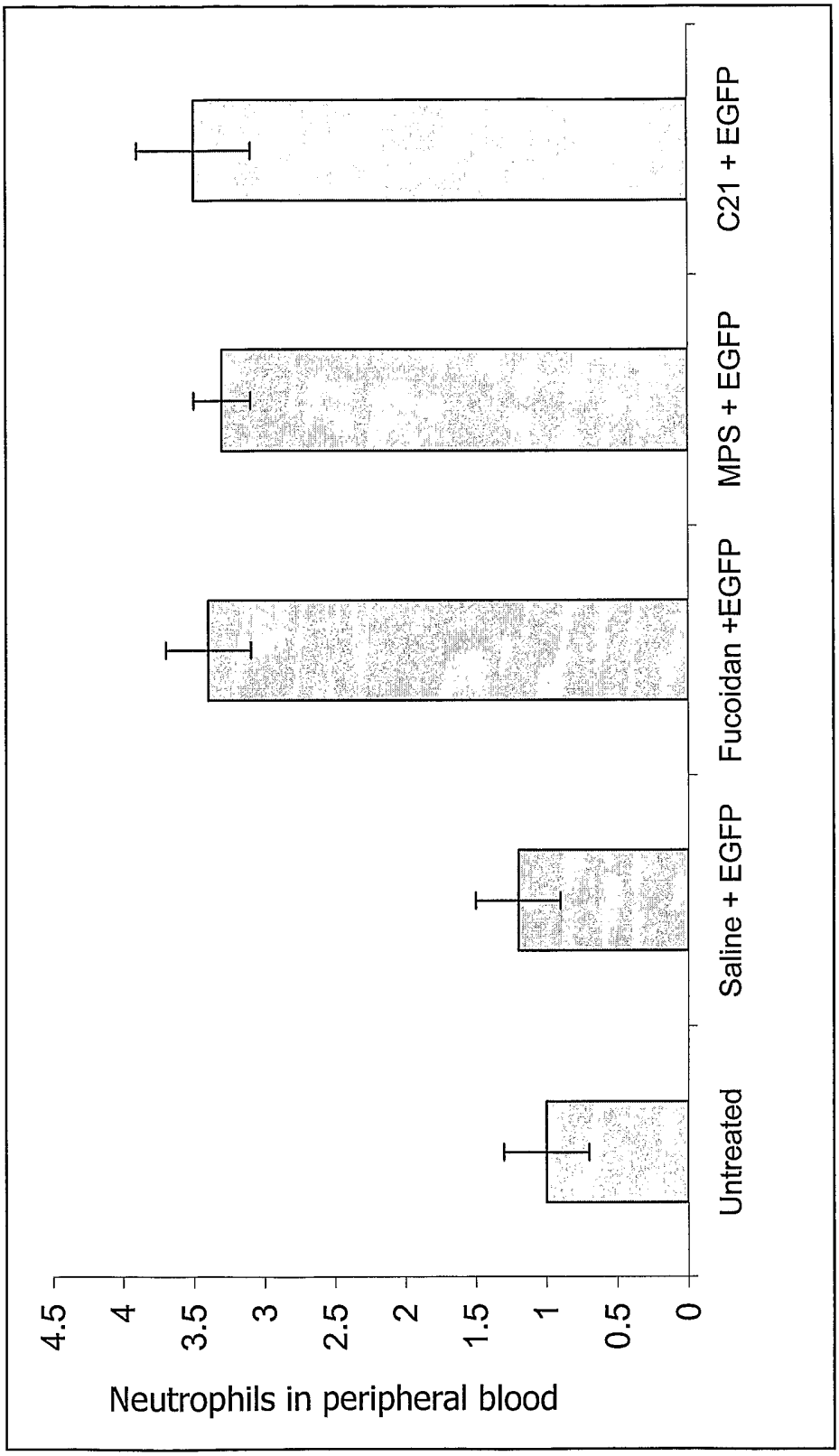

Neutrophils in peripheral blood
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Untreated
Saline + EGFP
Fucoidan +EGFP
MPS + EGFP
C21 + EGFP

Figure 6B

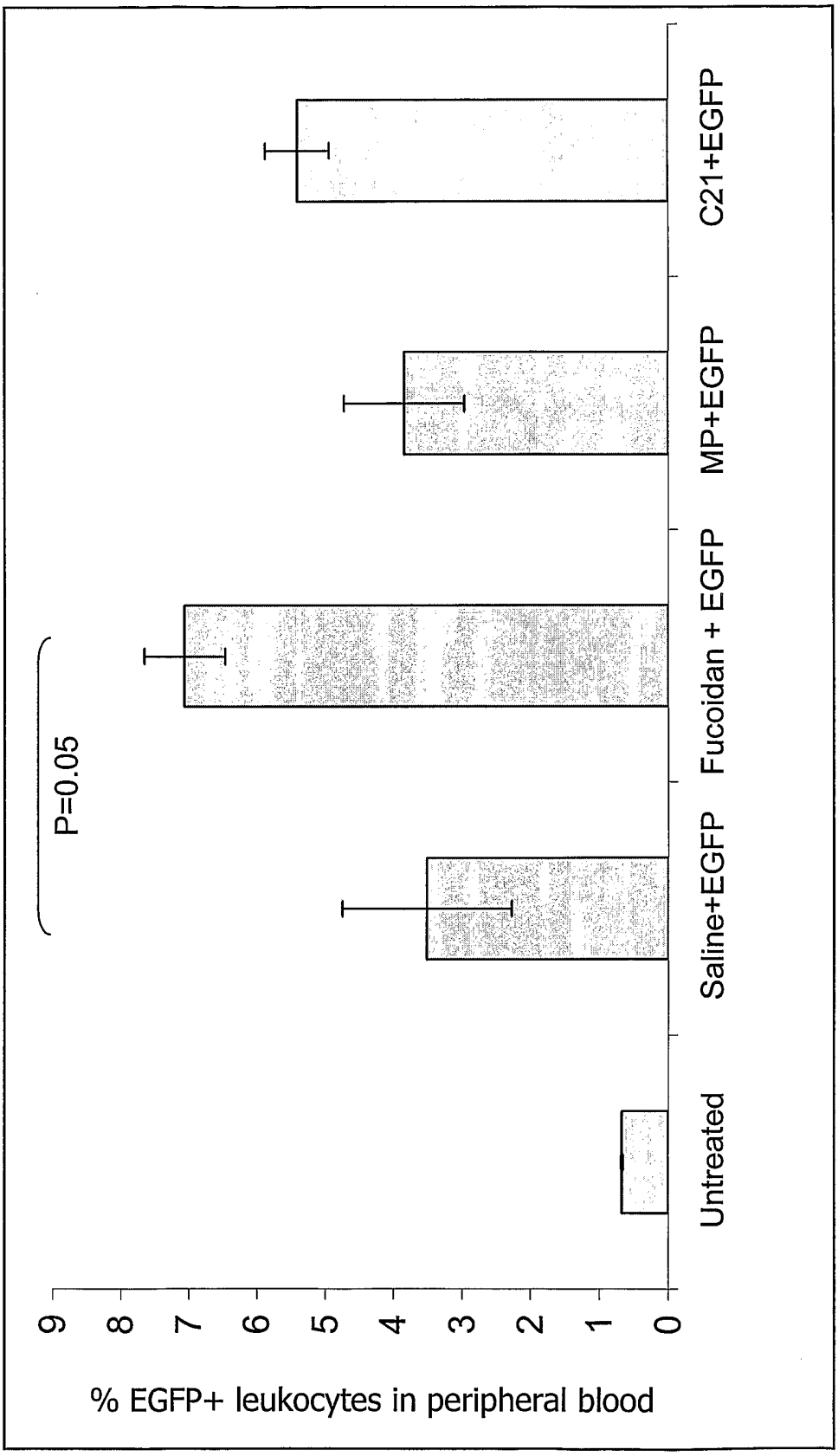

% EGFP+ leukocytes in peripheral blood
0
1
2
3
4
5
6
7
8
9
P=0.05
Untreated
Saline+EGFP
Fucoidan + EGFP
MP+EGFP
C21+EGFP

Figure 6C

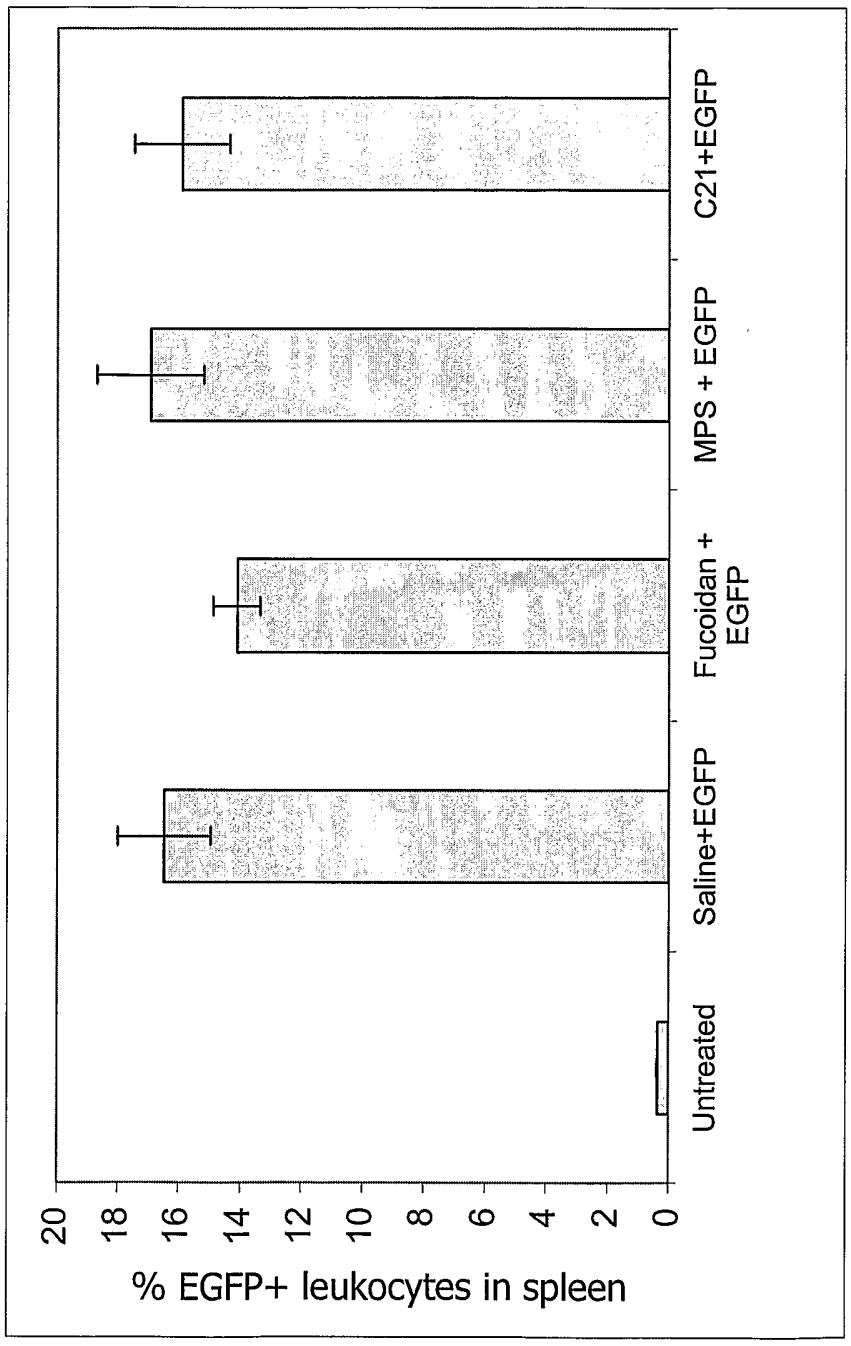

% EGFP+ leukocytes in spleen
0
2
4
6
8
10
12
14
16
18
20
Untreated
Saline+EGFP
Fucoidan + EGFP
MPS + EGFP
C21+EGFP

Figure 6D

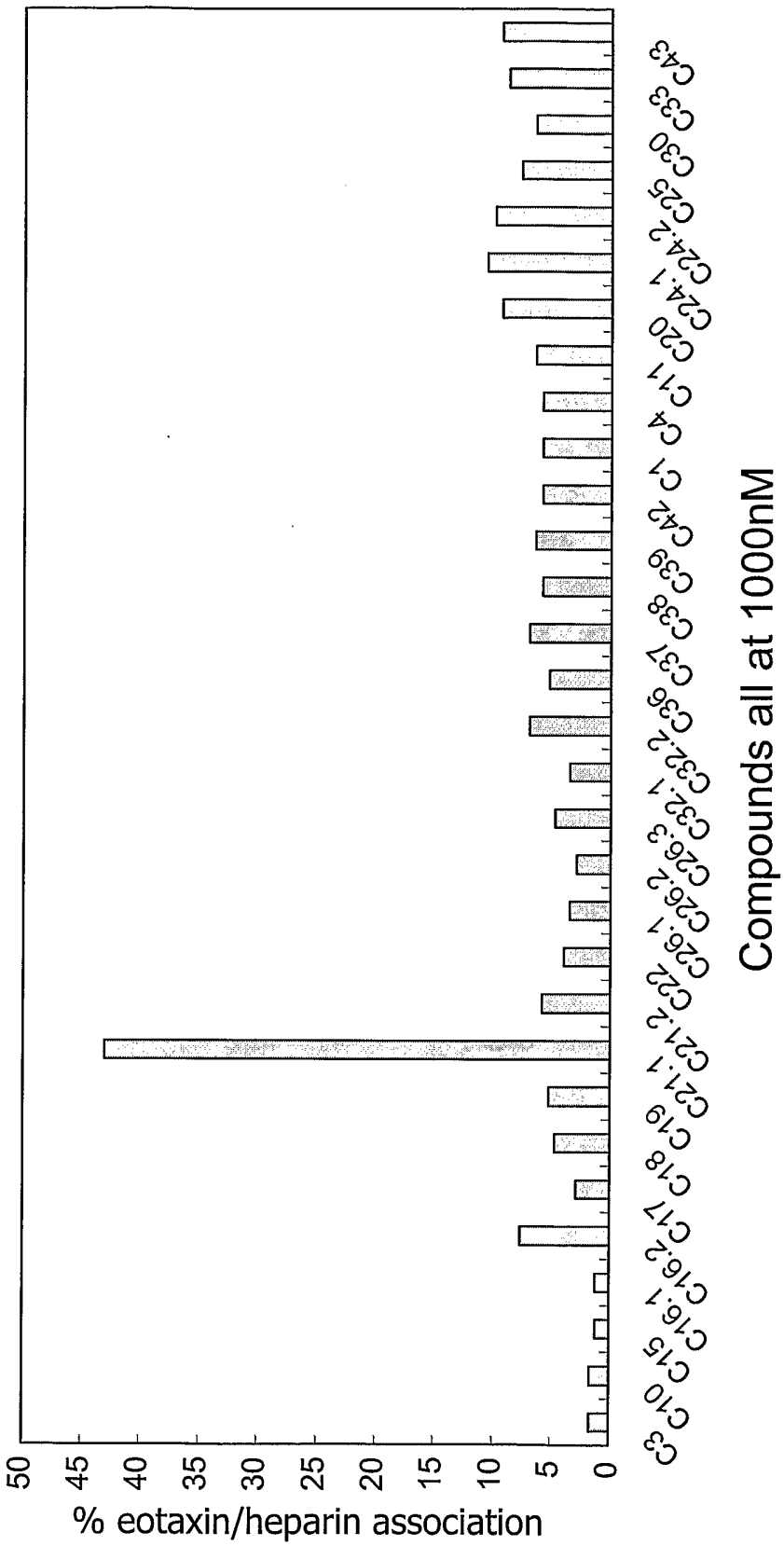

% eotaxin/heparin association
0
5
10
15
20
25
30
35
40
45
50
Compounds all at 1000nM
C3
C10
C15
C16.1
C16.2
C17
C18
C19
C21.1
C21.2
C22
C26.1
C26.2
C26.3
C32.1
C32.2
C36
C37
C38
C39
C42
C1
C4
C17
C20
C24.1
C24.2
C25
C30
C33
C43

Figure 7A

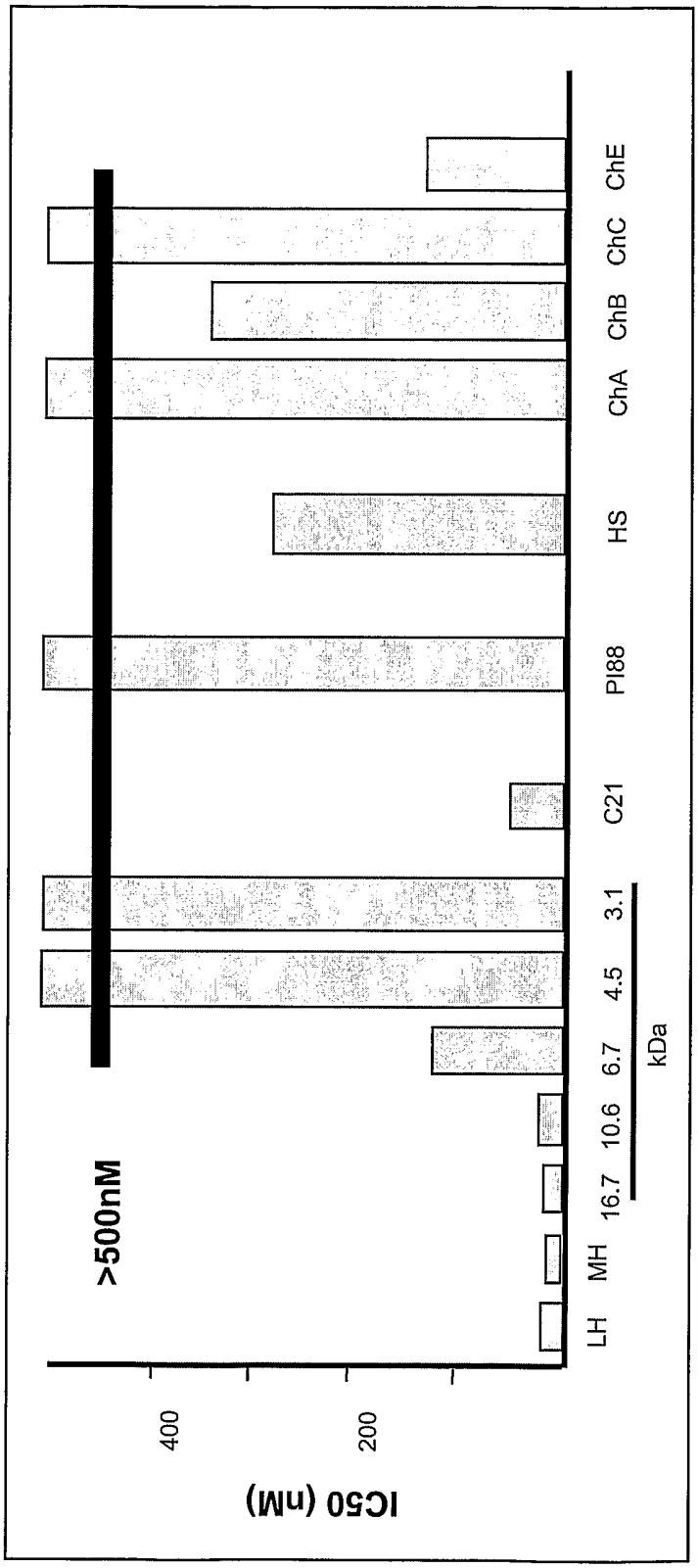

IC50 (nM)
400
200
>500nM
LH
MH
16.7
10.6
6.7
4.5
3.1
kDa
C21
PI88
HS
ChA
ChB
ChC
ChE

Figure 7B

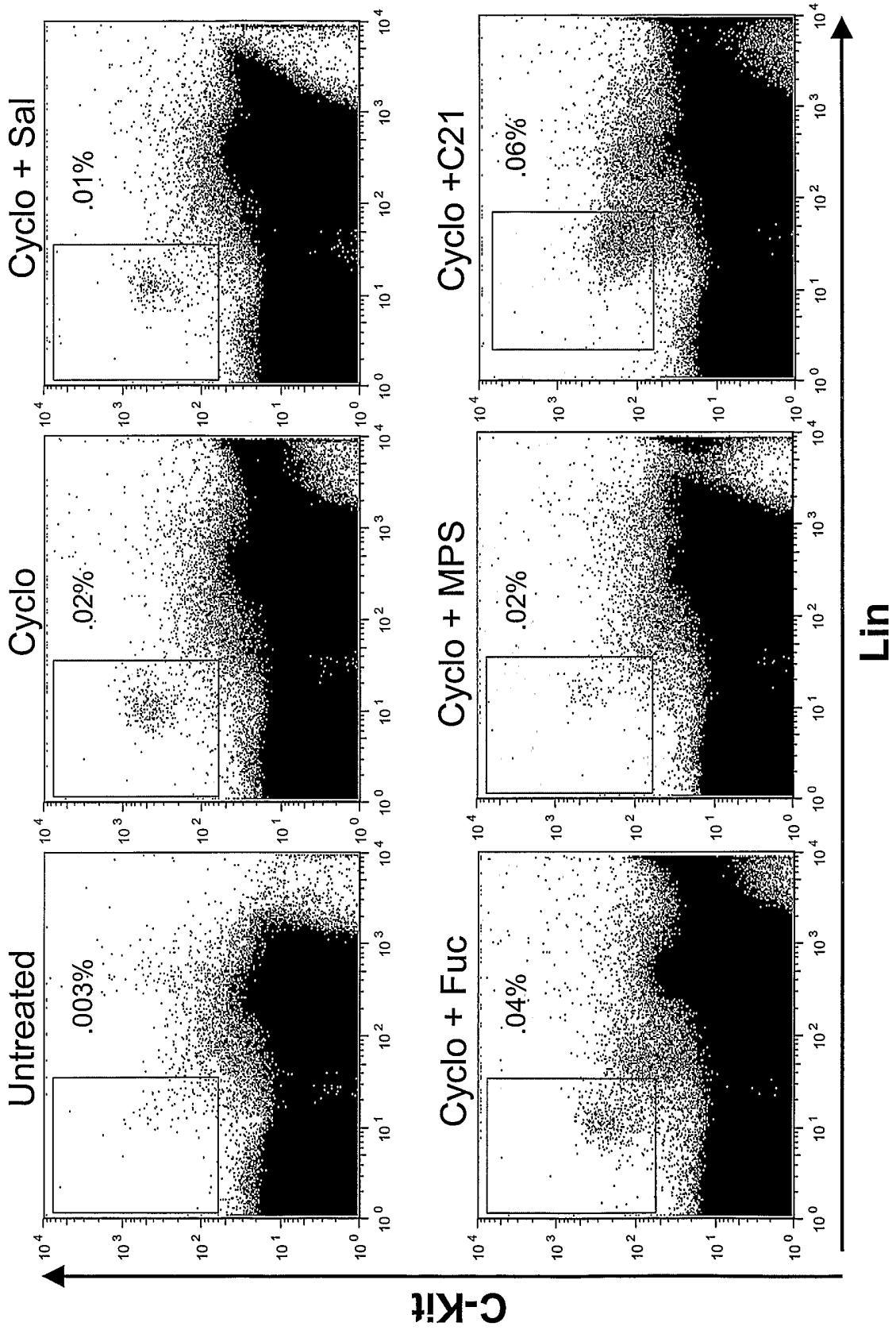

Untreated
.003%
Cyclo
.02%
Cyclo + Sal
.01%
Cyclo + Fuc
.04%
Cyclo + MPS
.02%
Cyclo +C21
.06%
c-Kit
Lin

Figure 8A

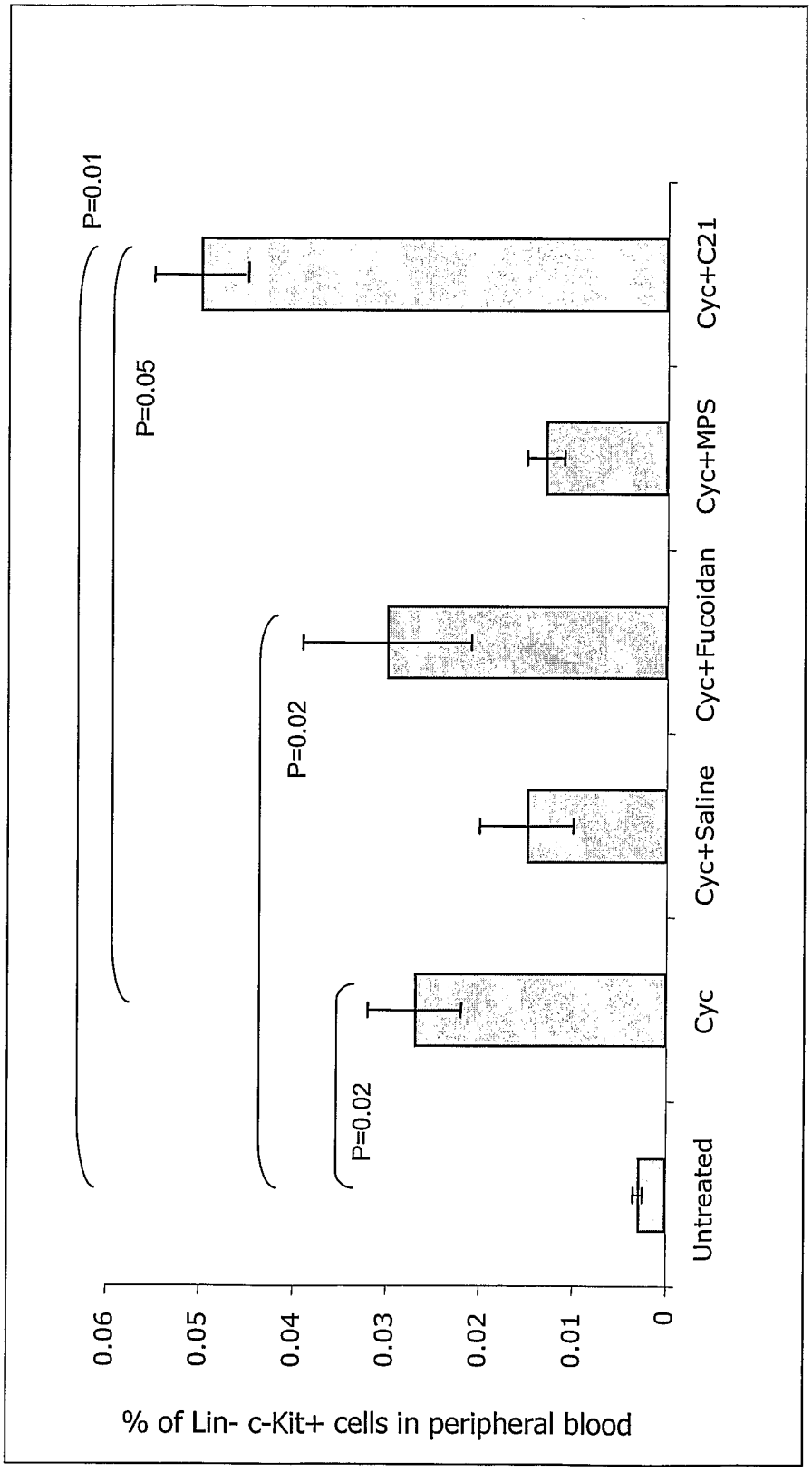

P=0.01
P=0.05
P=0.02
P=0.02
Untreated
Cyc
Cyc+Saline
Cyc+Fucoidan
Cyc+MPS
Cyc+C21
0
0.01
0.02
0.03
0.04
0.05
0.06
% of Lin- c-Kit+ cells in peripheral blood

Figure 8B

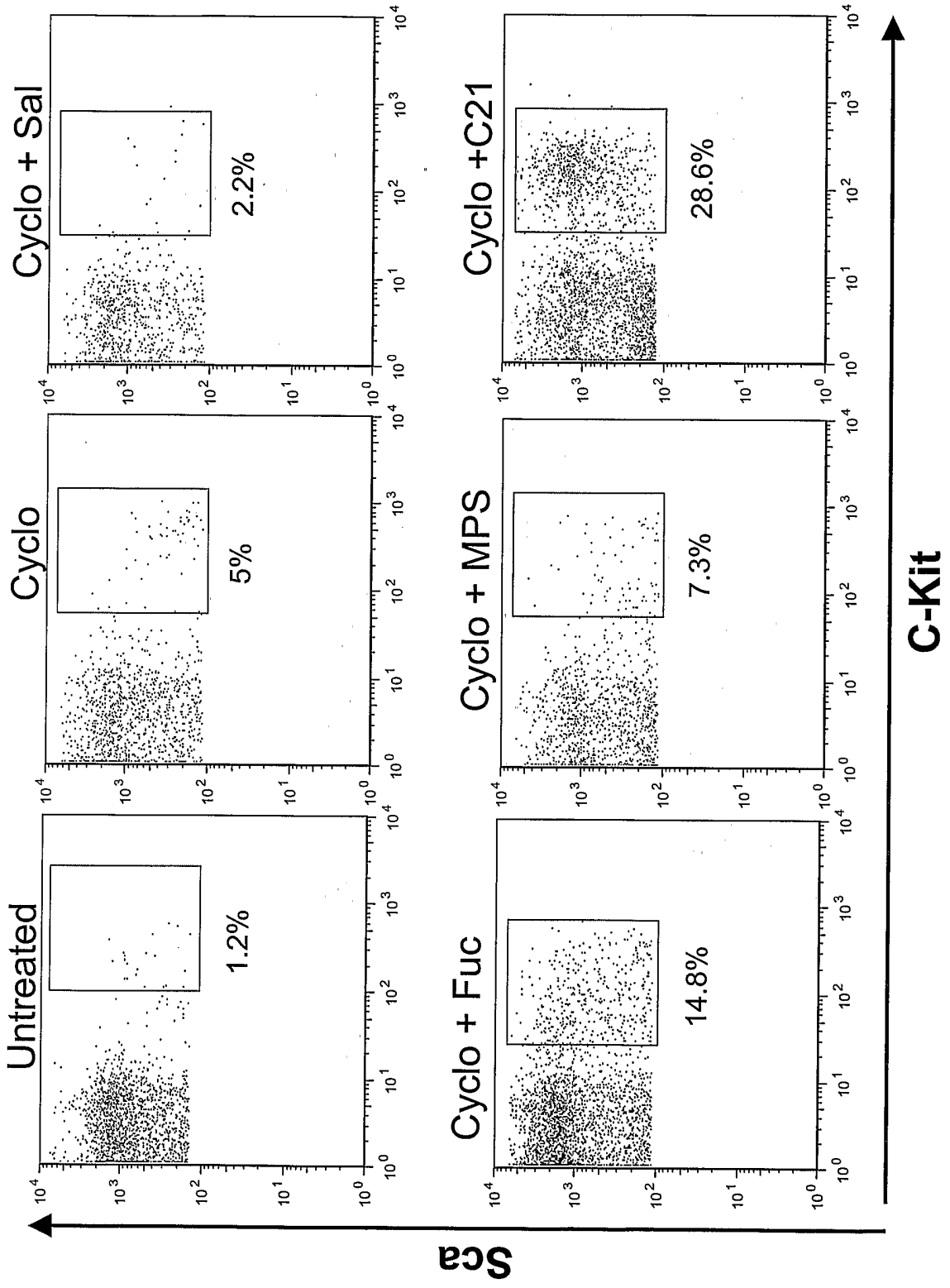

Untreated
1.2%
Cyclo
5%
Cyclo + Sal
2.2%
Cyclo + Fuc
14.8%
Cyclo + MPS
7.3%
Cyclo +C21
28.6%
Sca
C-Kit

Figure 9A

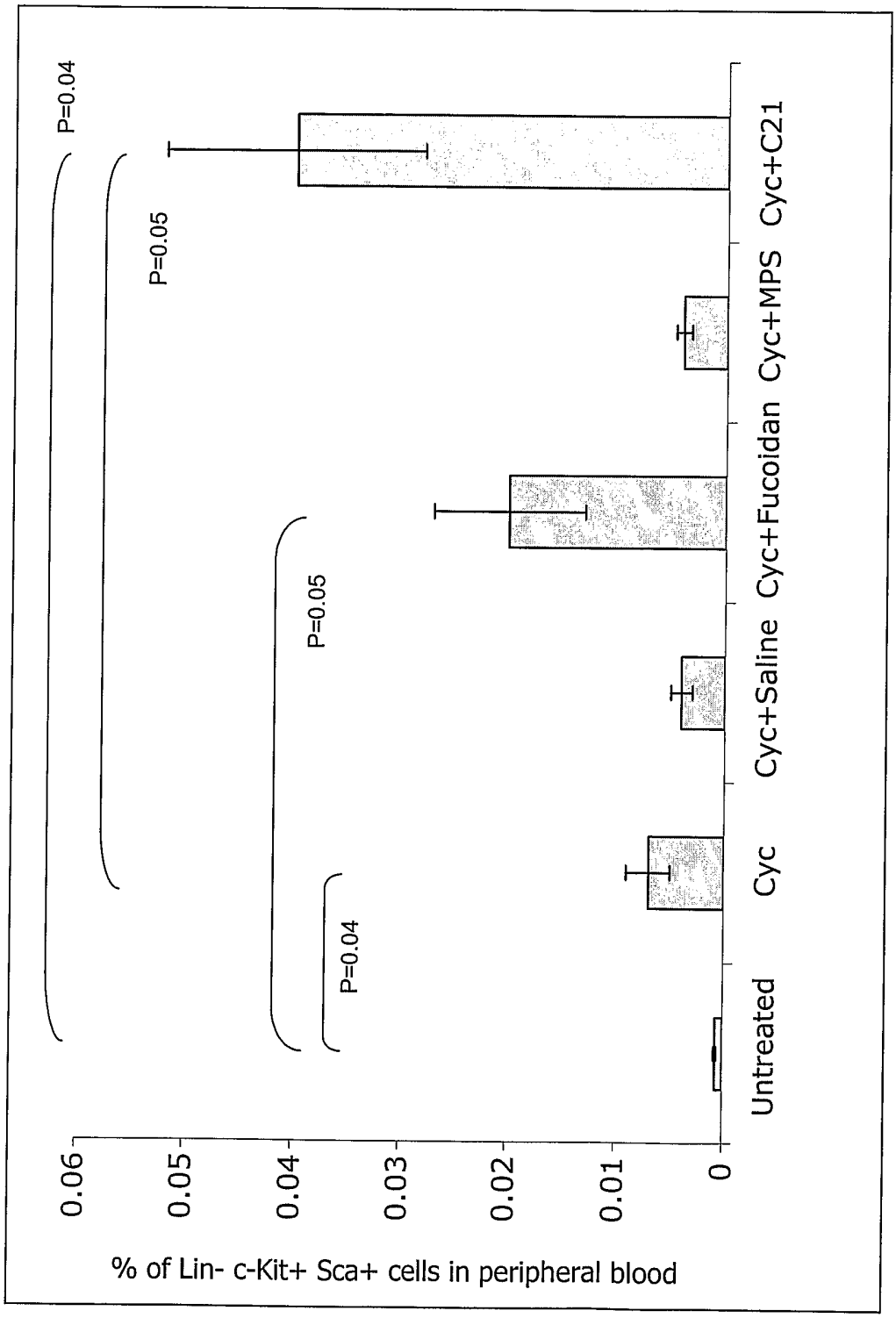

P=0.04
P=0.05
P=0.05
P=0.04
Untreated
Cyc
Cyc+Saline
Cyc+Fucoidan
Cyc+MPS
Cyc+C21
0
0.01
0.02
0.03
0.04
0.05
0.06
% of Lin- c-Kit+ Sca+ cells in peripheral blood

Figure 9B

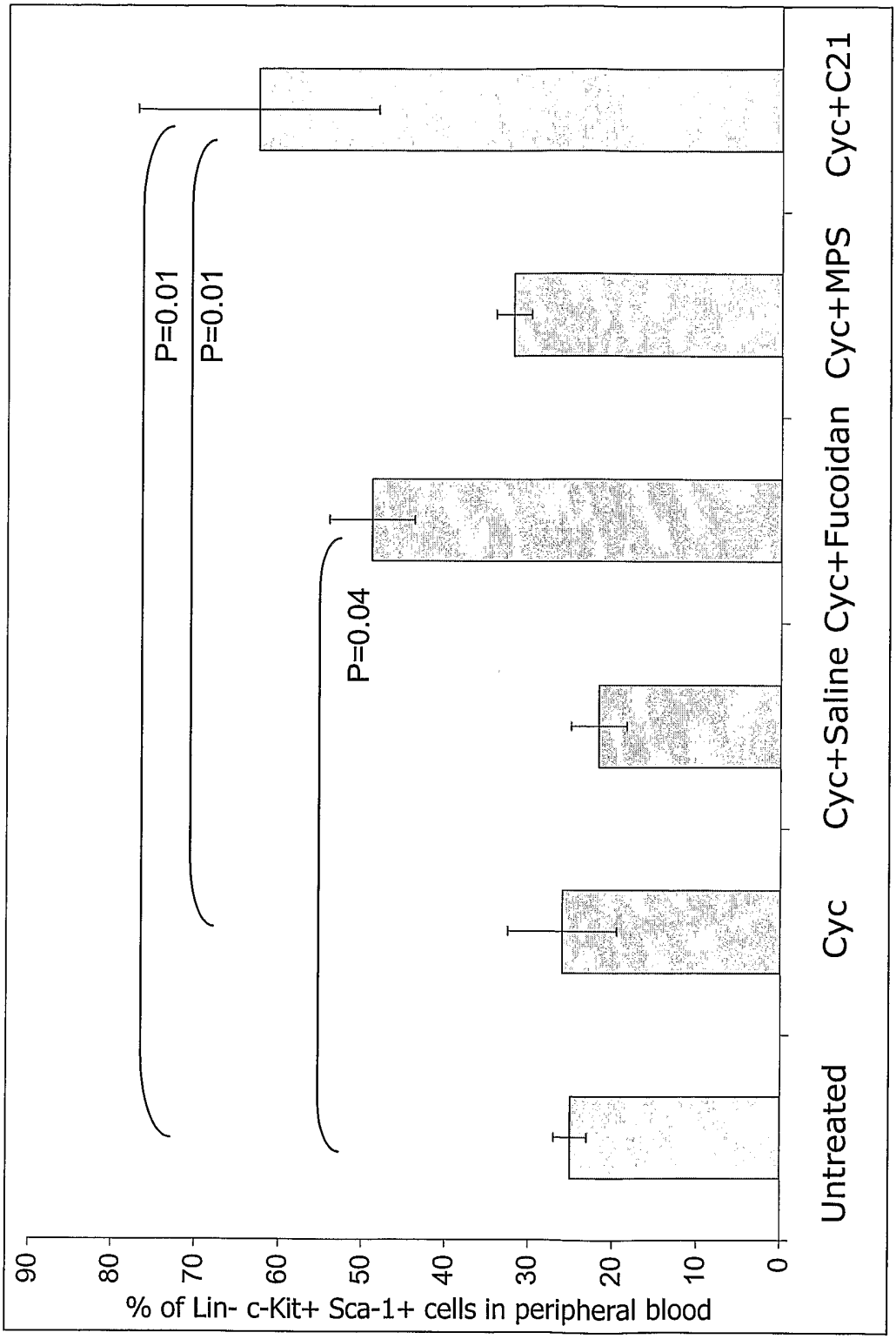

% of Lin- c-Kit+ Sca-1+ cells in peripheral blood
0
10
20
30
40
50
60
70
80
90
P=0.01
P=0.01
P=0.04
Untreated
Cyc
Cyc+Saline
Cyc+Fucoidan
Cyc+MPS
Cyc+C21

Figure 9C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2007/000209

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. *A61K 31/702* (2006.01) *A61K 31/724* (2006.01) *A61K 31/727* (2006.01) *A61P 43/00* (2006.01) *A61P 37/06* (2006.01)

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)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPIDS, CAPLUS & MEDLINE: stem cell and related terms, leukocyte, platelet, erythrocyte, saccharide, glycan, dextran and related terms, mobilise and related terms, cyclophosphamide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HIDALGO et al. The Integrin αMβ2 anchors hematopoietic progenitors in the bone marrow during enforced mobilization. Blood 2004, vol. 104(4) pages 993-1001 See whole document, especially abstract and introduction	1-4, 6, 9, 11, 16, 51
X	SWEENEY et al. Mobilization of stem/progenitor cells by sulfated polysaccharides does not require selectin pressure. PNAS 2000, vol. 97(12) pages 6544-6549. See whole document especially figures 5 and 6	1-4, 9, 11, 12, 16, 34-36, 51
X	SWEENEY et al. Sulfated polysaccharides increase plasma levels of SDF-1 in monkeys and mice: involvement in mobilization of stem/progenitor cells. Blood 2002, vol. 99(1), pages 44-51. See whole document especially pages 44, 46, 49	1-3, 9, 11, 12, 16, 51

[X] Further documents are listed in the continuation of Box C [ ] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"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)

"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

"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

"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

"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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 March 2007	19 APR 2007
Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	**NESRIN OZSARAC** Telephone No : (02) 6283 7958

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2007/000209

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SBAA-KETATA et al. Hyaluronan-derived oligosaccharides enhance SDF-1-dependent chemotactic effect on peripheral blood hematopoietic CD34+ cells. Stem Cells 2002, vol. 20, pages 585-587. See whole document.	1-3, 17, 51
A	VAN DER HAM et al. Mobilization of B and T lymphocytes and haemopoietic stem cells by polymethacrylic acid and dextran sulphate. Cells and Tissue Kinetics 1977, vol. 10(4), pages 387-397. See whole document	1-51
A	SWEENEY et al. Increase in circulating SDF-1 after treatment with sulfated glycans. Annals of the New York Academy of Sciences 2001, vol. 938, pages 48-53. See whole document	1-51
PX	ALKHATIB et al. Low molecular weight fucan prevents transplant coronapathy in rat cardiac allograft model. Transplant immunology 2006, vol. 16, pages 14-19. See whole document	1-51

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2007/000209

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of 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. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: **35-50 (in part)**
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:
These claims were searched in so far as they are limited by the technical feature of the ability to mobilize the stem cells. The search was limited to compositions disclosed in the preferred embodiments, claims 35-50 have not been searched across their entire scope as they are not limited to the technical feature that is their "ability to mobilize cells".

3. ☐ Claims Nos.:
because they are dependent 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:

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 claims Nos.:

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 claims 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.