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COMPOSITIONS AND METHODS USING UNBOUND MPL RECEPTOR FOR STIMULATING PLATELET PRODUCTION

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(56) Prior Art Documents
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(57) Claim

1. A method for increasing the number of platelets in a mammal, which comprises administering to the mammal a platelet number increasing effective amount of an unbound MPL receptor.

5. A method according to Claim 2, wherein said soluble MPL receptor is selected from the group consisting of polypeptides having a sequence of from residue 19 ($\pm$ 10 amino acids) to residue 483 ($\pm$ 10 amino acids) of SEQ. ID NO. 2.

6. A method according to Claim 2, wherein said soluble MPL receptor is selected from the group consisting of polypeptides having a sequence of from residue 26 ($\pm$ 10 amino acids) to residue 491 ($\pm$ 10 amino acids) of SEQ. ID NO. 4.

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(54) Title: COMPOSITIONS AND METHODS USING UNBOUND MPL RECEPTOR FOR STIMULATING PLATELET PRODUCTION (57) Abstract Disclosed is a method for increasing the number of platelets in a mammal, which comprises administering to the mammal a platelet number increasing effective amount of an unbound, preferably a soluble, MPL receptor.		

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Compositions and methods using unbound MPL receptor for stimulating platelet production.

Field of the Invention

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The present invention relates to the stimulation and growth of cells, particularly megakaryocytes and platelets, and specifically to the use of the so-called MPL receptor as an inducer of megakaryocyte differentiation into platelets. The present invention also relates to compositions capable of bringing about such platelet production *in vivo*.

Background of the Invention

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At least two broad areas of research are involved in the present invention. The first relates to the development and production of platelets from megakaryocytes and the second relates to a polypeptide member of a growth factor receptor family, referred to herein as the MPL receptor. Each of these areas of research will now be outlined in the following.

A. Platelet Production from Megakaryocytes

25

Blood platelets are circulating cells that are crucial for the prevention of bleeding and for blood coagulation. Megakaryocytes are the cellular source of platelets and arise from a common bone marrow precursor cell which gives rise to all hematopoietic cell lineages. This common precursor cell is known as the pluripotent stem cell or PPSC.

A hierarchy of megakaryocytic progenitor cells has been defined based on the time of appearance and size of megakaryocyte (MK) colonies appearing in in

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vitro culture systems in response to appropriate growth factors. The burst-forming unit megakaryocyte (BFU-MK) is the most primitive megakaryocyte progenitor cell. BFU-MK are thought to ultimately produce numerous colony forming unit megakaryocytes (CFU-MK), which are more differentiated MK progenitor cells.

As the MK undergo subsequent differentiation, MK cells lose the ability to undergo mitosis but acquire an ability to endoreduplicate. Endoreduplication is the phenomenon in cells of nuclear division in the absence of cell division. Endoreduplication ultimately results in an MK which is polyploid. Further MK maturation results in acquisition of cytoplasmic organelles and membrane constituents that characterize platelets.

Platelets are produced from mature MK's by a poorly defined process that has been suggested to be a consequence of MK physical fragmentation, or other mechanisms. Observations of extensive membranous structures within megakaryocytes has led to a model of platelet formation in which a demarcation membrane system outlines nascent platelets within the cell body. Another model of platelet formation has developed from observations that megakaryocytes will form long cytoplasmic processes constricted at platelet-sized intervals from which platelets presumably break off due to blood flow pressures in the marrow and/or in the lung. These cytoplasmic processes were termed proplatelets by Becker and DeBruyn to reflect their presumed precursor role in platelet formation. See Becker and DeBruyn, Amer. J. Anat. 145: 183 (1976).

FIG. 1 presents an overview of the various precursor cells involved in megakaryocyte and platelet development. The cell at the far left-hand side of the

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figure may be considered a PPSC, and the additional cells to the right of the PPSC in the figure may be thought of as BFU-MK, followed by CFU-MK. The cell that is undergoing endomitosis, which is located immediately to the right of the PPSC in the figure, is a mature megakaryocyte cell. As a result of endomitosis, this cell has become polyploid. The next structure to the right includes the long cytoplasmic processes that are constricted at platelet-sized intervals emerging from the polyploid nucleus of the mature megakaryocyte cell. In the far right-hand side of the figure are shown a number of platelets that have been produced by fragmentation of the cytoplasmic processes in the immediately preceding megakaryocyte cell.

15

The following is a summary of some prior publications relating to the above description of megakaryocyte maturation and the production of platelets:

20

1. Williams, N. and Levine, R.F., British Journal of Haematology 52: 173-180 (1982).
2. Levin, J., Molecular Biology and Differentiation of Megakaryocytes, pub. Wiley-Liss, Inc.: 1-10 (1990).
- 25 3. Gewirtz, A.M., The Biology of Hematopoiesis, pub. Wiley-Liss, Inc.: 123-132 (1990).
- 30 4. Han, Z.C., et al., Int J. Hematol. 54: 3-14 (1991).
5. Nieuwenhuis, H.K. and Sixma, J., New Eng. J. of Med. 327: 1812-1813 (1992).
- 35 6. Long, M., Stem Cells 11: 33-40 (1993).

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B. Regulation of Platelet Formation

A large body of data generated in many laboratories indicates that platelet production is regulated by humoral factors. The complexity of this biological process was not originally appreciated and currently it appears that a number of human growth factors possess this capability.

Megakaryocyte regulation occurs at multiple cellular levels. A number of cytokine cell proliferation factors amplify platelet production by expanding the progenitor cell pool. A second group of humoral growth factors serves as maturation factors acting on more differentiated cells to promote endoreduplication. In addition, there appear to be two independent biofeedback loops regulating these processes.

Several lineage nonspecific hematopoietic growth factors exert an important effect on MK maturation. Granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-3 (IL-3), IL-6, IL-11, leukemia inhibitory factor (LIF), and erythropoietin (EPO) each individually promote human MK maturation *in vitro* as determined by their effects on MK size, number, or ploidy. The MK maturational effects of LIF, IL-6, and IL-11 are either partially (LIF and IL-6) or totally (IL-11) additive to those of IL-3. Such data suggest that combinations of cytokines may be necessary to promote MK maturation *in vivo*.

The following is a summary of some prior publications relating to the regulation of megakaryocyte and platelet production:

- 5 -

7. Hoffman, R. et al., Blood Cells 13: 75-86 (1987).
8. Murphy, M.J., Hematology/Oncology Clinics of North America 3 (3): 465-478 (1988).
- 5 9. Hoffman, R., Blood 74 (4): 1196-1212 (1989).
10. Mazur, E.M. and Cohen, J.L., Clin. Pharmacol. Ther., 46 (3): 250-256 (1989).
- 10 11. Gewirtz, A.M. and Calabretta, B., Int. J. Cell Cloning 8: 267-276 (1990).
12. Williams, N., Progress in Growth Factor Research 2:
15 81-95 (1990).
13. Gordon, M.S. and Hoffman, R., Blood 80 (2): 302-307 (1992).
- 20 14. Hunt, P. et al., Exp. Hematol. 21: 372-281 (1993).
15. Hunt, P. et al., Exp. Hematol. 21: 1295-1304 (1993).

25

C. The MPL Receptor

The myeloproliferative leukemia virus (MPLV) is a murine replication-defective retrovirus that causes
30 acute leukemia in infected mammals. It has been discovered that a gene expressed by MPLV consists of a part of the gene that encodes the retroviral envelope (or external protein coat) of the virus fused to a sequence that is related to the cytokine receptor
35 family, including the receptors for GM-CSF, G-CSF, and EPO.

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Expression of the MPLV gene described above has the interesting biological property of causing murine progenitor cells of various types to immediately acquire growth factor independence for both

5 proliferation and terminal maturation. Moreover, some cultures of bone marrow cells acutely transformed by MPLV contained megakaryocytes, suggesting a connection between the MPLV gene and megakaryocyte growth and differentiation.

10

It is now recognized that the MPLV viral gene (referred to as v-MPL) has a homolog in mammalian cells, which is referred to as a cellular MPL gene (or c-MPL). Using v-MPL-derived probes, a cDNA corresponding to the
15 human c-MPL gene was cloned. See PCT published application WO 92/07074 (published April 30, 1992; discussed below). Sequence analysis has shown that the protein encoded by the c-MPL gene product belongs to the highly conserved cytokine receptor superfamily, just
20 like the homologous v-MPL gene product.

This cellular gene, c-MPL, is thought to play a functional role in hematopoiesis based on the observation that its expression was found in bone
25 marrow, spleen, and fetal liver from normal mice by Northern blot analysis, but not in other tissues. In particular, c-MPL is expressed on megakaryocytes. It has also been shown that the human cellular gene, human c-MPL, is expressed in purified megakaryocytes,
30 platelets and other cells that express the CD34 antigen, which is indicative of early hematopoietic progenitor cells. Furthermore, exposure of CD34 positive cells to synthetic oligodeoxynucleotides that are anti-sense to the c-MPL mRNA or message significantly inhibits the
35 colony forming ability of CFU-MK megakaryocyte

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progenitors, but has no effect on erythroid or granulomacrophage progenitors.

In total, the above data and observations
5 suggest that the c-MPL-encoded protein could be the
receptor for a cytokine specific for regulating
megakaryocytopoiesis. In other words, c-MPL appears to
encode a cell surface receptor, referred to herein as
the MPL receptor, that binds to a ligand, which
10 activates the receptor, possibly leading to production
of megakaryocytes.

PCT patent publication WO 92/07074 is directed
to the sequence of the protein produced by the c-MPL
15 gene, from both human and murine sources. This gene
product, which is thought to be a receptor as explained
above, is made up of at least three general regions or
domains: an extracellular domain, a transmembrane
domain, and an intracellular (or cytoplasmic) domain.
20 Attached together, these domains make up the intact MPL
receptor. This PCT publication also refers to a soluble
form of the receptor that substantially corresponds to
the extracellular domain of the mature c-MPL protein.
The intracellular domain contains a hydrophobic region
25 that, when attached via the transmembrane region to the
extracellular domain of the protein, renders the overall
protein subject to aggregation and insolubility. On the
other hand, when the extracellular domain of the c-MPL
gene product is separated from the transmembrane domain
30 and the intracellular domain, it becomes soluble, hence
the extracellular form of the protein is referred to as
a "soluble" form of the receptor.

A number of researchers are currently pursuing
35 the isolation and characterization of the putative
ligand which binds specifically to the c-MPL receptor.

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It is expected that such ligand will stimulate the production of mature megakaryocytes and/or platelets. To date, however, no one has reported the purification or final structure of such a ligand. On the other hand, according to the prior publications relating to the MPL receptor (i.e., the intact c-MPL gene product), the soluble form of the MPL receptor would be expected to bind to circulating free MPL ligand, thus inhibiting the formation of megakaryocytes and/or platelets.

10

The following is a summary of some prior publications relating to the above description of the v-MPL and c-MPL receptors and genes:

- 15 16. Wendling, F., et al., Leukemia 3 (7): 475-480 (1989).
17. Wendling, F., et al., Blood 73 (5): 1161-1167 (1989).
- 20 18. Souyri, M., et al., Cell 63: 1137-1147 (1990).
19. Vigon, I., et al., Proc. Natl. Acad. Sci. USA 89: 5640-5644 (1992).
- 25 20. Skoda, R.C., et al., The EMBO Journal 12 (7): 2645-2653 (1993).
21. Ogawa, M. Blood 81 (11): 2844-2853 (1993).
- 30 22. Methia, N., et al., Blood 82 (5): 1395-1401 (1993).
23. Wendling, F., et al., Blood 80: 246a (1993).

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D. The need for an agent capable of stimulating platelet production.

It has been reported recently that platelet
5 transfusions are being administered at an ever
increasing rate at medical centers in North America,
Western Europe, and Japan. See Gordon, M.S. and
Hoffman, R., Blood 80 (2): 302-307 (1992). This
increase appears to be due in large measure to advances
10 in medical technology and greater access to such
technologies as cardiac surgery and bone marrow, heart,
and liver transplantation. Dose intensification as a
means of delivering therapies to cancer patients and the
HIV-1 epidemic have also contributed to the heavy demand
15 on the platelet supply.

Platelet usage carries with it the possibility
of transmission of the many blood-born infectious
diseases as well as alloimmunization. Moreover, the
20 production of purified platelets is an expensive
endeavor and hence the increasing use of such platelets
increases overall medical costs. As a result, there
exists an acute need for new and improved methods for
producing platelets for human uses.

25

Exemplary prior approaches to enhancing
platelet production are described in the following:

U.S. patent 5,032,396 reports that
30 interleukin-7 (IL-7) is capable of stimulating platelet
production. Interleukin-7 is also known as
lymphopoietin-1 and is a lymphopoietic growth factor
capable of stimulating growth of B- and T-cell
progenitors in bone marrow. Published PCT application
35 serial number 88/03747, filed October 19, 1988 and
European patent application number 88309977.2, filed

October 24, 1988 disclose DNA's, vectors, and related processes for producing mammalian IL-7 proteins by recombinant DNA technology. The data presented in the U.S. patent show that IL-7 can increase circulating platelets in normal and sublethally irradiated mice.

5 U.S. patent 5,087,448 discloses that megakaryocytes and platelets can be stimulated to proliferate in mammals by treating them with interleukin-6. Recombinant human interleukin-6 is a 26,000 molecular weight glycoprotein with multiple biological activities. The data presented in this patent show that IL-6 has an effect of increasing colonies of megakaryocytes *in vitro*.

10 Neither of the above-cited patents mentions anything with respect to the MPL receptor, which is involved in the present invention.

In spite of the above disclosures, there remains a strong need for new stimulators of megakaryocytes and/or platelets in mammals.

It is an object of the present invention to provide a means of stimulating
15 production of megakaryocytes and/or platelets in a mammal.

SUMMARY OF THE INVENTION

According to a first aspect the present invention consists in a method for increasing the number of platelets in a mammal, which comprises administering to the mammal a platelet number increasing effective amount of an unbound MPL receptor.



These and other objects of the present invention as will hereinafter be described in greater detail, have been achieved by the discovery by the present inventors that administration of an unbound MPL receptor (defined below) to a mammal, results in an increased number of platelets in such mammal. The present invention also provides compositions for inducing platelet production, comprising an effective quantity of an unbound MPL receptor in admixture with one or more pharmaceutically acceptable diluents, carriers or excipients, as well as methods of using an unbound MPL receptor to prepare pharmaceutical compositions for inducing and enhancing platelet production in mammals.

15

Brief Description of the Figures

Numerous features and advantages of the present invention will become apparent upon review of the figures, wherein:

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FIG. 1 depicts an overview of development and maturation of megakaryocytes and platelets.

FIG. 2 shows a schematic comparison of the domains of MPL-P (human) and Mpl (murine). Additionally, a schematic depiction of an exemplary soluble form of the receptor is shown (Mpl-S).

25

FIG. 3 shows data demonstrating that the addition of soluble MPL receptor (MPL-X) to



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megakaryocyte cultures enhances the development of proplatelet formations.

FIG. 4 presents data showing the effect of
5 MPL-X *in vivo* on platelet counts in a mouse model.

FIG. 5 presents data showing the effect of
MPL-X *in vivo* on white blood cell (WBC) counts in a
mouse model.

10

FIG. 6 presents data showing the effect of
MPL-X *in vivo* on red blood cell (RBC) counts in a mouse
model.

15

Detailed Description of the Invention

Additional aspects and advantages of the
invention will be apparent to those skilled in the art
20 upon consideration of the following detailed
description, which details the practice of the
invention.

The present invention is based on the
25 unexpected discovery that MPL receptor, in unbound form,
is capable of stimulating an increase in the number of
platelets *in vivo* in mammals. This is believed to be
due to an increased rate of megakaryocyte fragmentation
into platelets. While it was previously appreciated
30 that the MPL receptor might have some involvement in
megakaryo-cytopoiesis, the understanding in the art was
that when the receptor was bound to a megakaryocyte
cell, it required stimulation by a ligand to cause the
megakaryocyte to mature and/or to produce platelets.
35 Based on the above hypothesis, it was a logical
expectation that MPL receptor not attached to a cell

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surface (i.e., "unbound MPL receptor") would compete for ligand with the MPL receptor bound to the cell, thereby removing ligand that would normally stimulate the cell to produce megakaryocytes and platelets, with the final
5 effect being fewer cells proceeding to produce megakaryocytes and platelets.

The present inventors, however, have discovered that the opposite of the above-described
10 expected result is actually observed. That is, in *in vivo* experiments in mammals, unbound MPL receptor has the effect of enhancing the production of megakaryocytes and platelets.

15 While not wishing to be bound by any particular mechanism or theory of action, the present inventors have formulated a hypothesis which may explain the effects described herein. This hypothesis is that the ligand that normally stimulates the bound MPL
20 receptor produces mature megakaryocytes from less mature cells (e.g., PPSC, BFU-MK, CFU-MK), but that once these mature megakaryocytes are produced, in order to proceed to form proplatelets, the ligand must be removed. If the ligand is not removed from the mature megakaryocyte,
25 this megakaryocyte remains in the mature form rather than producing platelets.

Using the above hypothesis as a model, the administration of unbound MPL receptor would serve to
30 allow mature megakaryocytes to proceed to form proplatelets and then fragment into platelets. At the same time, removal of the MPL ligand by the unbound MPL receptor may also inhibit the formation of additional mature megakaryocytes. If this hypothetical model is
35 correct, it leads to the conclusion that even greater platelet formation might be achieved upon administration

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of both the ligand and the unbound MPL receptor, since such administration would result in both enhanced production of mature megakaryocytes as well as stimulation of the production of platelets from the mature megakaryocytes. Sequential administration of ligand followed by unbound receptor might even further enhance the effect. In any case, the present inventors believe that this discovery is completely unexpected in view of the prior models of platelet formation and MPL receptor/ligand activity.

Definitions

The following definitions explain the meaning of key terms and phrases in the claims herein.

By "increasing the number of platelets" is meant that the number of platelets is significantly elevated above the normal range of platelets in the particular mammal involved. The elevation of platelet counts may occur in a time-dependent manner, and may be cyclical, increasing and then constant or decreasing, or constant, etc.

For example, with reference to FIG. 4, it appears that there are at least three cycles of elevated platelet count, with the first occurring at about seven to eight days, the second and more enhanced cycle occurring at between nineteen and twenty days, and the third cycle occurring at about twenty-eight to twenty-nine days. These cycles occurred in several mice in a particular model system, and may or may not be duplicated in other mammalian systems or models.

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Preferably, the elevation in platelet count will be at least about forty to fifty percent above the average of the normal range, but may be considerably higher, such as up to about a two-fold increase as compared to the normal average number of platelets. It should be born in mind that any significant increase, such as, for example, five to ten percent, might be clinically sufficient in a given situation. The increase in platelet count is a function of, among other variables, the dose of the unbound MPL receptor administered to the mammal, and so can be altered over a wide range.

Platelets may be counted by any of various standard methods. One exemplary method involves the use of a commercially available blood cell counter. Such instruments "count" particles and classify them into cell types based essentially on size. Instruments such as the Sysmex or the Coulter Counter are exemplary. For a discussion of such methods, see Bloom, A.L. and Thomas, D.P. (eds.), Haemostasis and Thrombosis (Second Edition) Churchill Livingstone: 936 (1987).

The "mammal" to be treated is not specifically limited, but is preferably a human. Other mammals that might be treated include dogs, cats, cows, horses, etc.. Cross-species activity (i.e., activity of an MPL receptor from one species in another species) is also expected, especially if there is a high (e.g., equal to or above around 80%) homology between the MPL receptors from the two species, and is therefor another aspect of the present invention. For example, murine unbound MPL receptor could be used to increase the number of platelets in a human. However, it is expected that the greatest activity will be achieved using the same species' MPL receptor as the species being treated.

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"Administration" may be carried out by any convenient method. For example, bolus injection, continuous infusion, sustained release from implants, or any other suitable technique could be used. Intravenous
5 administration is preferred. Multiple administrations of unbound MPL receptor are also contemplated.

A "platelet number increasing effective amount" is an amount sufficient to significantly raise
10 the number of platelets in a particular mammal. If necessary or desired, this amount can be appropriately determined in preliminary trials, without undue experimentation, and depending upon the level of increase desired in the mammal being treated.
15 Generally, doses of 1 μ g to 100 μ g per kilogram per day, preferably 10 μ g to 100 μ g per kilogram per day for one to twenty days can be expected to induce a therapeutically significant biological effect. The unbound MPL receptor may be administered each day for a
20 period of days, all at once on a first day followed by one or more days without administration, etc. Preferably, bolus injections of 10 μ g per kilogram per day can be given at 3 - day intervals as a stimulator of platelet production.

25

In addition to the unbound MPL receptor, one or more additional lymphokines or cytokines may be administered simultaneously or sequentially with the MPL receptors of the present invention. Such lymphokines
30 and/or cytokines may be, for example, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-11, colony stimulating factor-1 (CSF-1), GM-CSF, granulocyte colony stimulating factor (G-CSF), EPO, interferon-alpha (IFN-alpha), IFN-beta, or IFN-gamma. It is also possible to
35 include the MPL ligand once it is available. Any of

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these may work additively or synergistically with the unbound MPL receptors.

By "unbound MPL receptor" is meant an MPL
5 receptor molecule that is free from the ordinary cellular constituents, especially the cell membrane. Preferably, the unbound receptor will be the so-called "soluble" form of the receptor. The "soluble" form of the MPL receptor may be any such form known to or
10 subsequently developed by those skilled in the art. Generally, the soluble form of the receptor will be one which substantially lacks the transmembrane domain of the receptor molecule.

15 It is known that the transmembrane domain of the MPL receptor is very hydrophobic, and therefore it is highly preferred that substantially all of this domain of the MPL receptor be removed for purposes of this invention. Although the intracellular domain of
20 the MPL receptor is not considered insoluble, it is nevertheless preferred that this domain also be substantially removed from the MPL receptor for purposes of this invention.

25 In summary, by "soluble" is meant that the material is not bound to a cell, and preferably includes substantially none (i.e., fewer than about 10 residues) of the transmembrane or intracellular domains of the full-length molecule. The soluble MPL receptor (which
30 is also referred to herein as MPL-X) is capable of being dissolved in aqueous solution as well as body fluids, such as blood, serum, saliva, cerebrospinal fluid, urine, etc. It is also possible to use the extracellular domain of the MPL receptor directly
35 attached to the intracellular domain of the receptor. As used herein, "MPL receptor" preferably refers to the

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cellular form of this receptor (i.e., the c-MPL gene product) rather than the virally-derived form of the gene (i.e., v-MPL).

5 The intact MPL receptor and its constituent domains are described in detail in published PCT publication WO92/07074, published April 30, 1992. Further details on the murine form of the MPL receptor are found in Vigon, et al., EMBO 8: 2607 - 2615 (1993).
10 In addition, SEQ. ID NOS. 1 and 2 herein present the full length sequences of the murine MPL receptor gene and protein, respectively, while SEQ. ID NOS. 3 and 4 present the full length sequences of the murine MPL receptor gene and protein, respectively.

15

 In the murine sequence (SEQ. ID NO. 2), the domains are as follows:

signal peptide	aa 1 (Met) through aa 18 (Ser)
20 extracellular	aa 19 (Gln) through aa 483 (Trp)
transmembrane	aa 484 (Ile) through aa 505 (Leu)
cytoplasmic	aa 506 (Lys) through aa 626 (Pro)

Referring again to the murine sequence (SEQ. ID NO. 2),
25 some preferred soluble sequences are:

aa 19 (± 10 aa) through aa 483 (± 10 aa), and
aa 19 (± 10 aa) through aa 484 (± 10 aa) fused to
30 aa 506 (± 10 aa) through aa 626 (± 10 aa).

 It is known that the human sequence has at least two forms, MPL-P and MPL-K. The P form has the amino acid and gene sequences shown in SEQ. ID NOS. 3
35 and 4, respectively. In the human MPL-P sequence, the domains are as follows:

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signal peptide	aa 1 (Met) through aa 25 (Ser)
extracellular	aa 26 (Gln) through aa 491 (Trp)
transmembrane	aa 492 (Ile) through aa 513 (Leu)
cytoplasmic	aa 514 (Arg) through aa 635 (Pro)

5

Referring to the human sequence (SEQ. ID NO. 4), some preferred soluble sequences are:

aa 19 (± 10 aa) through aa 483 (± 10 aa), and

10

aa 19 (± 10 aa) through aa 484 (± 10 aa) fused to
aa 506 (± 10 aa) through aa 626 (± 10 aa).

With reference to FIG. 2, one example of a
15 soluble form of the receptor is depicted (Mpl-S). It
might be possible to remove even a larger portion of
cytokine receptor domain II (CRD II) from either of the
human or murine sequences and still have a soluble form
of the receptor with platelet enhancing activity. Thus,
20 the present inventors also contemplate the use of
soluble receptors wherein substantially all of the CRD
II domain is removed from the MPL receptor to enhance
platelet production in a patient.

25 As exemplified above, based on the known MPL
sequences and the prior publications in this area or
routine experimentation, one of ordinary skill will be
able to readily obtain a family of unbound murine or
human MPL receptors for use in the context of this
30 invention.

Various biologically active analogs of the
foregoing proteins could also be employed in the methods
and compositions of the present invention. As used
35 herein, therefore, the term "MPL receptor" includes
proteins having substantial amino acid sequence identity

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to native mammalian MPL receptor and qualitatively equivalent biological activity, for example, in standard bioassays (e.g., assays of the receptor binding affinity or antibody binding affinity of the analog protein).

5 Particularly preferably, the analogs of the unbound MPL receptor of the present invention are based on the soluble form of the protein. The MPL receptor may further be in pegylated form or fused to other cytokine proteins or fragments thereof, e.g., IL-3, IL-6, IL-11,
10 GM-CSF, EPO, and the like.

Preferred methods for producing mammalian MPL receptors of the present invention involve recombinant expression in mammalian cells, although such proteins
15 can also be produced recombinantly using insect cells, yeast, bacteria, or other cells under the control of appropriate promoters. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by
20 Powels et al., Cloning Vectors: A Laboratory Manual (Elsevier New York, 1985). Various mammalian cell culture systems can be employed to express recombinant protein.

25 Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell 23: 175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, 293, HeLa and BHK cell
30 lines. Mammalian expression vectors may comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome
35 binding sites; a polyadenylation site; splice donor and acceptor sequences; and termination sequences. DNA

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sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites, may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. Additional details regarding the use of mammalian high expression vectors to produce a recombinant mammalian MPL receptor are provided below. Exemplary vectors can be constructed as disclosed by Okayama and Berg, Mol. Cell. Biol. 3:280 (1983); Cosman et al., Nature 312:768 (1984); Cosman et al., Mol. Immunol. 23:935 (1986); and Clark et al., U.S. Pat. No. 4,675,285.

The conditions to be treated by the methods and compositions of the present invention are generally those which involve an existing platelet deficiency or an expected platelet deficiency in the future (e.g., because of planned surgery). The generic term for platelet deficiency is thrombocytopenia, and hence the methods and compositions of the present invention are generally available for treating thrombocytopenias.

Thrombocytopenias (platelet deficiencies) may be present for various reasons, including chemotherapy, radiation therapy, surgery, accidental blood loss, and other specific disease conditions. Exemplary specific disease conditions that involve thrombocytopenia and may be treated in accordance with this invention are: aplastic anemia, idiopathic thrombocytopenia, and certain metastatic tumors which result in thrombocytopenia. Also, certain treatments for AIDS result in thrombocytopenia (e.g., AZT). Certain wound healing disorders might also benefit from an increase in platelet numbers.

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With regard to anticipated platelet deficiencies, e.g., due to future surgery, the MPL receptor could be administered several days to several hours prior to the need for platelets. With regard to
5 acute situations, e.g., accidental and massive blood loss, the MPL receptor could be administered along with blood or purified platelets.

The specific dosage amount of unbound MPL
10 receptor to be administered in connection with these conditions will be affected by a number of variables, including: the weight, sex, age, and general condition of the patient to be treated; the nature of the condition to be treated; the mode of administration; the
15 urgency of the situation; and others. In acute situations, the dose to be administered will generally be larger than with chronic conditions or conditions with lesser platelet deficiency.

20 The compositions of the present invention include a "pharmaceutically acceptable carrier" in which the unbound receptor is soluble. The carrier material may be water for injection, preferably supplemented with other materials common in solutions for administration
25 to mammals. Typically, an MPL receptor therapeutic will be administered in the form of a composition comprising purified protein in conjunction with physiologically acceptable carriers, excipients, or diluents. Neutral buffered saline or saline mixed with serum albumin are
30 exemplary appropriate diluents. Preferably, the product is formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Other standard carriers, diluents, and excipients may be included as desired.

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EXAMPLES

The following examples are included to more fully illustrate the present invention. Additionally, these examples provide preferred embodiments of the invention, but are not meant to limit the scope thereof, unless so indicated. Standard methods for many of the procedures described in the following examples, or suitable alternative procedures, are provided in widely recognized manuals of molecular biology such as, for example, Sambrook et al., "Molecular Cloning," Second Edition, Cold Spring Harbor Laboratory Press (1987) and in Ausubel et al., (Eds), "Current Protocols in Molecular Biology," Greene associates/Wiley Interscience, New York (1990).

EXAMPLE 1

Isolation of nucleic acid sequences encoding an MPL receptor

The isolation of clones encoding the murine (Vigon et al. Oncogene 8:2607-2615 (1993) and (Skoda et al., EMBO) 12 (7): 2645-2653 (1993)) and human (Vigon et al., DNAS 89: 5640-5644 (1992)) isoforms of the MPL receptor have been reported. The DNA sequence of these are available from the GenBank database as accession numbers X73677 and 222657 for the murine receptor and M90102 and M90103 for the K and P isoforms of the human receptor. It has further been reported that spleen, bone marrow, placenta and fetal liver are all tissues which express mRNA for MPL. Cell lines which express MPL include HEL. It thus becomes clear to one skilled in the art, how to isolate clones encoding MPL. cDNA could be generated from one of the above tissue sources or cell line, or from another source identified to

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express MPL by Northern blot analysis of RNA or Western blot analysis of protein.

This cDNA serves as a source of material for the generation of a cDNA library in an appropriate vector. This library is screened by hybridization to a nucleic acid probe to identify cells containing the MPL gene. Alternatively the cDNA library is screened for expression of MPL using an antibody. This antibody is generated against a synthetic oligo-peptide corresponding to the MPL sequence.

The cDNA can also serve as a template for PCR amplification of the MPL gene. The primers are chosen from sequences in the MPL gene and may incorporate additional sequences to aid in the cloning and expression of MPL.

EXAMPLE 2

20

Isolation of clones for expression of soluble murine MPL

Using a clone containing MPL as identified above, or cDNA from a source capable of expressing MPL, the PCR technique is used to obtain a clone for expression of soluble MPL. Primers for PCR amplification of murine MPL may be of the form:

5' primer:

30 TAC AAG CTT GCC GTC ATC ATG CCC TCT TGG GCC CTC
(SEQ. ID NO. 5); and

3' primer:

ACT TCT AGA CTA TCA AGC AGT CTC GGA GCT GGA
35 (SEQ. ID NO. 6)

- 25 -

PCR reactions are carried out using 1 μ l of a cDNA reaction mix, 5 pmol of each of the above oligonucleotides, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM Mg Cl₂, 200 μ M each dNTP and 1 unit of Tag polymerase. Amplification is for 35 cycles of 30 sec. at 94°C, 30 sec. at 50°C, 1 min at 72°C. DNA is then purified by agarose gel electrophoresis, digested with Hind III and XbaI and ligated into the expression vector pDSR α 2 digested with Hind III and XbaI. Clones containing the desired insert are verified by DNA sequence analysis.

EXAMPLE 3

15 Expression of soluble murine MPL in CHO cells

The expression plasmid pDSR α 2-MPL-X contains sequences encoding murine MPL amino acids as shown in (SEQ. ID NOS. 7 and 8). Ten micrograms of this plasmid were introduced into CHO cells by calcium phosphate mediated transfection (Wigler et al., Cell 11: 233 (1977)). Individual colonies were selected based upon expression of the dihydrofolate reductase gene from the vector. Expression of soluble MPL was monitored by RNA hybridization (Hunt et al., Exp. Hematol, 19: 779 (1991)) and by Western blotting using an antibody generated to a synthetic peptide corresponding to amino acids 459 (Gly) to 475 (Val) of SEQ. ID NO. 2. Cell line B.1-18 was positive in these assays and was selected for further expansion. The cell line was adapted to 30nM Methotrexate (Mtx) to stimulate amplification of MPL expression. Roller bottles were inoculated with 2×10^7 cells in 200 ml DMEM: Ham's F12 (1:1) supplemented with non-essential amino acids (NEAA), 30nM Mtx and 5% fetal bovine serum (FBS)

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(reagents from GIBCO, Grand Island, NY). Upon reaching confluence in 3-4 days, the media was replaced with 200 ml DMEM: Ham's F12, NEAA, 30 nM Mtx with no serum. Conditioned media was harvested after 6-7 days and
5 replaced with fresh serum-free media. Second and third harvests were collected.

EXAMPLE 4

10 Purification of soluble murine MPL

The recombinant, extracellular domain of the murine MPL gene product (m-MPL-X) was purified from CHO cell conditioned media by sequential ion exchange and
15 hydroxyapatite chromatography. Conditioned media containing m-MPL-X was concentrated 10X by ultrafiltration with a 30,000 MW cutoff membrane (S10Y30, Amicon, Danvers, MA) and diafiltered against 10 mM Tris-HCL, pH 8.5. The concentrate was loaded onto a
20 column of Q-Sepharose, fast flow (Pharmacia, Piscataway, NJ), washed with 10 mM Tris-HCL, pH 8.5, and eluted with a linear gradient of 0 M-1.0 M NaCl in the same buffer. Fractions from the column were analyzed for m-MPL-X by SDS-PAGE and Western blotting, using an antiserum
25 generated against purified m-MPL-X. Fractions containing m-MPL-X were pooled, diafiltered against 10 mM sodium phosphate, 0.01 mM CaCl₂, pH 6.8, and applied to a hydroxyapatite column (HA-Ultragel, Sepracor, Malborough, MA). The unbound fraction, containing
30 purified m-MPL-X was diluted into PBS, sterile filtered, and aseptically dispensed in appropriate volumes at a final concentration of 0.10 mg/ml. The purified m-MPL-X was stored frozen at -80° C until use.

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The Limulus Amebocyte Lysate assay (Associates of Cape Cod, Inc., Woods Hole, MA) was performed, and the final product was shown to be free of pyrogen (< 0.004 EU/mg). Analysis of 20 µg of purified m-MPL-X by SDS-PAGE with Coomassie staining demonstrated the presence of a single band with apparent molecular weight of 64 KD. No other protein bands were detected.

10

EXAMPLE 5

The effect of MPL-X on proplatelet formation

Guinea pig megakaryocytes were purified and cultured at 5000 cells per well (96- well microtiter plate) with or without MPL-X (30 µg/ml; prepared as described above) for 18 hours. The basal media was Iscove's media supplemented either with BSA (100 µg/ml) or with 10% normal human heparinized plasma (pooled from AB donors). After culture, cells were examined microscopically and scored for proplatelet formations as described by Hunt et al. See Hunt, P. et al., Exp. Hematol. 21: 372-281 (1993) and Hunt, P. et al., Exp. Hematol. 21: 1295-1304 (1993). Data is presented as the mean of duplicate determinations +/- standard error of the mean (SEM).

Guinea pig megakaryocytes cultured in serum-free media or in heparinized plasma have previously been shown to develop proplatelet formations after 18 hours of culture. See Hunt, P. et al., Exp. Hematol. 21: 372-281 (1993) and Hunt, P. et al., Exp. Hematol. 21: 1295-1304 (1993). As shown in FIG. 3, the addition of MPL-X to megakaryocyte cultures resulted in a significant increase in the number of cells developing proplatelet

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formations. This was observed whether the cells were cultured under serum-free conditions or in the presence of heparinized plasma.

5

EXAMPLE 6

The effect of MPL-X on *in vivo* proplatelet formation

10 MPL-X was diluted to 100 µg/ml or 10 µg/ml into carrier (PBS + 0.2% normal Balb/c mouse sera). In addition, 100 µg/ml of bovine serum albumin (BSA) or heat-inactivated MPL-X (98°C; 15 minutes) were used. Balb/c mice (female, 6-8 weeks of age, Charles River)
15 were injected subcutaneously twice daily, with an 8 hour interval, with 0.5 ml of each test solution for up to 42 days. On the indicated days, animals were bled from the lateral tail vein through a small incision made with a scalpel blade. Twenty microliters of blood were
20 collected and diluted immediately into manufacturer's diluent for the Sysmex cell analyzer (TOA Medical Electronics, Kobe, Japan). White blood cell (WBC), red blood cell (RBC), and platelet counts were obtained. Data are expressed as the mean of the indicated number of
25 samples +/- SEM.

In order to determine directly the effect of MPL-X on platelet levels, the protein was injected twice daily into normal Balb/c mice. The data from two separate experiments were combined for presentation. In
30 Experiment 1, five mice per group were injected either with carrier, 10 µg/day MPL-X, or 100 µg/day MPL-X for 42 days. In Experiment 2, four mice per group were injected either with carrier, 100 µg/day MPL-X, 100 µg/day heat-inactivated MPL-X or 100 µg/day BSA for 29
35 days. Whenever data from both experiments were collected from the same time point, they were combined

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for analysis (N=9, days 0, 4, 7, 11, 18; N=4 or 5, all other days). As shown in FIG. 4, injection of 100 µg/day MPL-X resulted in a significant increase in platelet counts. The effect was first observed after 4-7 days of treatment where platelet levels reached 132% normal levels. Although by Day 11 platelet counts had returned to normal, with continued administration of MPL-X, they again rose reaching 219% of normal by Day 18. After 20 days of treatment, the platelet counts had dropped to 150% of normal. When the study was terminated at 42 days, platelet counts were still 130% of normal. As also shown in FIG. 4, administration of 10 µg/day of MPL-X, 100 µg/day of BSA or heat inactivated MPL-X had no effect on platelet counts. MPL-X administration had no effect on other blood cell parameters. Figures 5 and 6 illustrate the data on white blood cell counts and red blood cell counts.

These data indicate that the administration of MPL-x *in vivo* results in a significant increase in circulating platelets. The response appears to be cyclic with a periodicity of approximately 7-10 days. No other blood cell parameter was affected at any time point measured.

* * *

While the present invention has been described above both generally and in terms of preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art in light of the above description. Therefore, it is intended that the appended claims cover all such variations coming within the scope of the invention as claimed.

- 30 -

Additionally, the publications and other materials cited to illuminate the background of the invention, and in particular cases to provide additional details concerning its practice, are herein incorporated
5 by reference.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 5 (i) APPLICANTS: Choi, Esther S.
Hokom, Martha M.
Hunt, Pamela
Nichol, Janet L.
- 10 (ii) TITLE OF INVENTION: Compositions And Methods For
Stimulating Platelet Production
- 15 (iii) NUMBER OF SEQUENCES: 8
- (iv) CORRESPONDENCE ADDRESS:
20 (A) ADDRESSEE: Amgen Inc., U.S. Patent Operations/PRC
(B) STREET: 1840 DeHavilland Drive
(C) CITY: Thousand Oaks
(D) STATE: CA
(F) ZIP: 91320-1789
- 25 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- 30 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- 35 (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 805-447-4955

(2) INFORMATION FOR SEQ ID NO:1:

- 40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2046 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
45 (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: cDNA
- 50 (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 8..1888

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Met Val Thr Ser Cys Leu Leu Leu Ala Leu Pro Asn Gln Ala
1 5 10

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5	CAA GTC ACC AGC CAA GAT GTC TTC TTG CTG GCC TTG GGC ACA GAG CCC	97
	Gln Val Thr Ser Gln Asp Val Phe Leu Leu Ala Leu Gly Thr Glu Pro	
	15 20 25 30	
10	CTG AAC TGC TTC TCC CAA ACA TTT GAG GAC CTC ACC TGC TTC TGG GAT	145
	Leu Asn Cys Phe Ser Gln Thr Phe Glu Asp Leu Thr Cys Phe Trp Asp	
	35 40 45	
15	GAG GAA GAG GCA GCA CCC AGT GGG ACA TAC CAG CTG CTG TAT GCC TAC	193
	Glu Glu Glu Ala Ala Pro Ser Gly Thr Tyr Gln Leu Leu Tyr Ala Tyr	
	50 55 60	
20	CGA GGA GAG AAG CCC CGT GCA TGC CCC CTG TAT TCC CAG AGT GTG CCC	241
	Arg Gly Glu Lys Pro Arg Ala Cys Pro Leu Tyr Ser Gln Ser Val Pro	
	65 70 75	
25	ACC TTT GGA ACC CGG TAT GTG TGC CAG TTT CCA GCC CAG GTA GAA GTG	289
	Thr Phe Gly Thr Arg Tyr Val Cys Gln Phe Pro Ala Gln Val Glu Val	
	80 85 90	
30	CGC CTC TTC TTT CCG CTG CAC CTC TGG GTG AAG AAT GTG TCC CTC AAC	337
	Arg Leu Phe Phe Pro Leu His Leu Trp Val Lys Asn Val Ser Leu Asn	
	95 100 105 110	
35	CAG ACT TTG ATC CAG CGG GTG CTG TTT GTG GAT AGT GTG GGC CTG CCA	385
	Gln Thr Leu Ile Gln Arg Val Leu Phe Val Asp Ser Val Gly Leu Pro	
	115 120 125	
40	GCT CCC CCC AGG GTC ATC AAG GCC AGG GGT GGG AGC CAA CCA GGG GAA	433
	Ala Pro Pro Arg Val Ile Lys Ala Arg Gly Gly Ser Gln Pro Gly Glu	
	130 135 140	
45	CTT CAG ATC CAC TGG GAG GCC CCT GCT CCT GAA ATC AGT GAC TTC CTG	481
	Leu Gln Ile His Trp Glu Ala Pro Ala Pro Glu Ile Ser Asp Phe Leu	
	145 150 155	
50	AGG CAT GAA CTC CGC TAT GGC CCC ACG GAT TCC AGC AAC GCC ACT GCC	529
	Arg His Glu Leu Arg Tyr Gly Pro Thr Asp Ser Ser Asn Ala Thr Ala	
	160 165 170	
55	CCC TCC GTC ATT CAG CTG CTC TCC ACA GAA ACC TGC TGC CCC ACT TTG	577
	Pro Ser Val Ile Gln Leu Leu Ser Thr Glu Thr Cys Cys Pro Thr Leu	
	175 180 185 190	
60	TGG ATG CCG AAC CCA GTC CCT GTT CTT GAC CAG CCT CCG TGT GTT CAT	625
	Trp Met Pro Asn Pro Val Pro Val Leu Asp Gln Pro Pro Cys Val His	
	195 200 205	
65	CCG ACA GCA TCC CAA CCG CAT GGA CCA GTG AGG ACC TCC CCA GCT GGA	673
	Pro Thr Ala Ser Gln Pro His Gly Pro Val Arg Thr Ser Pro Ala Gly	
	210 215 220	
70	GAA GCT CCA TTT CTG ACA GTG AAG GGT GGA AGC TGT CTC GTC TCA GGC	721
	Glu Ala Pro Phe Leu Thr Val Lys Gly Gly Ser Cys Leu Val Ser Gly	
	225 230 235	

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	CTC CAG GCT AGC AAA TCC TAC TGG CTC CAG CTA CGC AGC CAA CCC GAC	769
	Leu Gln Ala Ser Lys Ser Tyr Trp Leu Gln Leu Arg Ser Gln Pro Asp	
	240 245 250	
5	GGG GTC TCC CTT CGT GGC TCC TGG GGA CCC TGG TCC TTC CCT GTG ACT	817
	Gly Val Ser Leu Arg Gly Ser Trp Gly Pro Trp Ser Phe Pro Val Thr	
	255 260 265 270	
10	GTG GAT CTT CCA GGA GAT GCA GTG ACA ATT GGA CTT CAG TGC TTT ACC	865
	Val Asp Leu Pro Gly Asp Ala Val Thr Ile Gly Leu Gln Cys Phe Thr	
	275 280 285	
15	TTG GAT CTG AAG ATG GTC ACC TGC CAG TGG CAG CAA CAA GAC CGC ACT	913
	Leu Asp Leu Lys Met Val Thr Cys Gln Trp Gln Gln Gln Asp Arg Thr	
	290 295 300	
20	AGC TCC CAA GGC TTC TTC CGT CAC AGC AGG ACG AGG TGC TGC CCC ACA	961
	Ser Ser Gln Gly Phe Phe Arg His Ser Arg Thr Arg Cys Cys Pro Thr	
	305 310 315	
25	GAC AGG GAC CCC ACC TGG GAG AAA TGT GAA GAG GAG GAA CCG CGT CCA	1009
	Asp Arg Asp Pro Thr Trp Glu Lys Cys Glu Glu Glu Glu Pro Arg Pro	
	320 325 330	
30	GGA TCA CAG CCC GCT CTC GTC TCC CGC TGC CAC TTC AAG TCA CGA AAT	1057
	Gly Ser Gln Pro Ala Leu Val Ser Arg Cys His Phe Lys Ser Arg Asn	
	335 340 345 350	
35	GAC AGT GTT ATT CAC ATC CTT GTA GAG GTG ACC ACA GCG CAA GGT GCC	1105
	Asp Ser Val Ile His Ile Leu Val Glu Val Thr Thr Ala Gln Gly Ala	
	355 360 365	
40	GTT CAC AGC TAC CTG GGC TCC CCT TTT TGG ATC CAC CAG GCT GTG CTC	1153
	Val His Ser Tyr Leu Gly Ser Pro Phe Trp Ile His Gln Ala Val Leu	
	370 375 380	
45	CTT CCC ACC CCG AGC CTG CAC TGG AGG GAG GTC TCA AGT GGA AGG CTG	1201
	Leu Pro Thr Pro Ser Leu His Trp Arg Glu Val Ser Ser Gly Arg Leu	
	385 390 395	
50	GAG TTG GAG TGG CAG CAC CAG TCA TCT TGG GCA GCT CAA GAG ACC TGC	1249
	Glu Leu Glu Trp Gln His Gln Ser Ser Trp Ala Ala Gln Glu Thr Cys	
	400 405 410	
55	TAC CAG CTC CGG TAC ACG GGA GAA GGC CGT GAG GAC TGG AAG GTG CTG	1297
	Tyr Gln Leu Arg Tyr Thr Gly Glu Gly Arg Glu Asp Trp Lys Val Leu	
	415 420 425 430	
60	GAG CCA TCT CTC GGT GCC CGG GGA GGG ACC CTA GAG CTG CGC CCC CGA	1345
	Glu Pro Ser Leu Gly Ala Arg Gly Gly Thr Leu Glu Leu Arg Pro Arg	
	435 440 445	
65	GCT CGC TAC AGC TTG CAG CTG CGT GCC AGG CTC AAC GGC CCC ACC TAC	1393
	Ala Arg Tyr Ser Leu Gln Leu Arg Ala Arg Leu Asn Gly Pro Thr Tyr	
	450 455 460	

- 34 -

	CAA GGT CCC TGG AGC GCC TGG TCT CCC CCA GCT AGG GTG TCC ACG GGC	1441
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	465 470 475	
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	Ser Glu Thr Ala Trp Ile Thr Leu Val Thr Ala Leu Leu Val Leu	
	480 485 490	
10	AGC CTC AGT GCC CTT CTG GGC CTA CTG CTG CTA AAG TGG CAA TTT CCT	1537
	Ser Leu Ser Ala Leu Leu Gly Leu Leu Leu Leu Lys Trp Gln Phe Pro	
	495 500 505 510	
15	GCG CAC TAC AGG AGA CTG AGG CAT GCT TTG TGG CCC TCG CTT CCA GAC	1585
	Ala His Tyr Arg Arg Leu Arg His Ala Leu Trp Pro Ser Leu Pro Asp	
	515 520 525	
20	CTA CAC CGG GTC CTA GGC CAG TAC CTC AGA GAC ACT GCA GCC CTA AGT	1633
	Leu His Arg Val Leu Gly Gln Tyr Leu Arg Asp Thr Ala Ala Leu Ser	
	530 535 540	
	CCT TCT AAG GCC ACG GTT ACC GAT AGC TGT GAA GAA GTG GAA CCC AGC	1681
	Pro Ser Lys Ala Thr Val Thr Asp Ser Cys Glu Glu Val Glu Pro Ser	
	545 550 555	
25	CTC CTG GAA ATC CTC CCT AAA TCC TCA GAG AGC ACT CCT TTA CCT CTG	1729
	Leu Leu Glu Ile Leu Pro Lys Ser Ser Glu Ser Thr Pro Leu Pro Leu	
	560 565 570	
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	Cys Pro Ser Gln Pro Gln Met Asp Tyr Arg Gly Leu Gln Pro Cys Leu	
	575 580 585 590	
35	CGG ACC ATG CCC CTG TCT GTG TGT CCA CCC ATG GCT GAG ACG GGG TCC	1825
	Arg Thr Met Pro Leu Ser Val Cys Pro Pro Met Ala Glu Thr Gly Ser	
	595 600 605	
	TGC TGC ACC ACA CAC ATT GCC AAC CAC TCC TAC CTA CCA CTA AGC TAT	1873
	Cys Cys Thr Thr His Ile Ala Asn His Ser Tyr Leu Pro Leu Ser Tyr	
	610 615 620	
40	TGG CAG CAG CCC TGAAGGCAGT CCCCATGCTA CTGCAGACCT ATACATTCCT	1925
	Trp Gln Gln Pro	
	625	
45	ACACACTACC TTATCCATCC TCAACACCAT CCATTCTGTT GCCACCCAC TCCCCGTCTG	1985
	GCTTTATAAC ACTGATCACT CCAAGATGGC TGCTCACAAA TCCAGAGCTC TGTCTCTGCA	2045
50	G	2046

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- 55 (A) LENGTH: 626 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

- 35 -

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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5   Met Val Thr Ser Cys Leu Leu Leu Ala Leu Pro Asn Gln Ala Gln Val
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    Thr Ser Gln Asp Val Phe Leu Leu Ala Leu Gly Thr Glu Pro Leu Asn
      20             25             30
10   Cys Phe Ser Gln Thr Phe Glu Asp Leu Thr Cys Phe Trp Asp Glu Glu
      35             40             45

    Glu Ala Ala Pro Ser Gly Thr Tyr Gln Leu Leu Tyr Ala Tyr Arg Gly
    50             55             60
15   Glu Lys Pro Arg Ala Cys Pro Leu Tyr Ser Gln Ser Val Pro Thr Phe
    65             70             75             80

    Gly Thr Arg Tyr Val Cys Gln Phe Pro Ala Gln Val Glu Val Arg Leu
      85             90             95
20   Phe Phe Pro Leu His Leu Trp Val Lys Asn Val Ser Leu Asn Gln Thr
      100            105            110

    Leu Ile Gln Arg Val Leu Phe Val Asp Ser Val Gly Leu Pro Ala Pro
    115            120            125
25   Pro Arg Val Ile Lys Ala Arg Gly Gly Ser Gln Pro Gly Glu Leu Gln
    130            135            140

    Ile His Trp Glu Ala Pro Ala Pro Glu Ile Ser Asp Phe Leu Arg His
    145            150            155            160
35   Glu Leu Arg Tyr Gly Pro Thr Asp Ser Ser Asn Ala Thr Ala Pro Ser
      165            170            175

    Val Ile Gln Leu Leu Ser Thr Glu Thr Cys Cys Pro Thr Leu Trp Met
      180            185            190
40   Pro Asn Pro Val Pro Val Leu Asp Gln Pro Pro Cys Val His Pro Thr
      195            200            205

    Ala Ser Gln Pro His Gly Pro Val Arg Thr Ser Pro Ala Gly Glu Ala
    210            215            220
45   Pro Phe Leu Thr Val Lys Gly Gly Ser Cys Leu Val Ser Gly Leu Gln
    225            230            235            240

    Ala Ser Lys Ser Tyr Trp Leu Gln Leu Arg Ser Gln Pro Asp Gly Val
    245            250            255
50   Ser Leu Arg Gly Ser Trp Gly Pro Trp Ser Phe Pro Val Thr Val Asp
      260            265            270

    Leu Pro Gly Asp Ala Val Thr Ile Gly Leu Gln Cys Phe Thr Leu Asp
    275            280            285
55

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Leu Lys Met Val Thr Cys Gln Trp Gln Gln Gln Asp Arg Thr Ser Ser
 290 295 300

5 Gln Gly Phe Phe Arg His Ser Arg Thr Arg Cys Cys Pro Thr Asp Arg
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Asp Pro Thr Trp Glu Lys Cys Glu Glu Glu Glu Pro Arg Pro Gly Ser
 325 330 335

10 Gln Pro Ala Leu Val Ser Arg Cys His Phe Lys Ser Arg Asn Asp Ser
 340 345 350

Val Ile His Ile Leu Val Glu Val Thr Thr Ala Gln Gly Ala Val His
 355 360 365

15 Ser Tyr Leu Gly Ser Pro Phe Trp Ile His Gln Ala Val Leu Leu Pro
 370 375 380

20 Thr Pro Ser Leu His Trp Arg Glu Val Ser Ser Gly Arg Leu Glu Leu
 385 390 395 400

Glu Trp Gln His Gln Ser Ser Trp Ala Ala Gln Glu Thr Cys Tyr Gln
 405 410 415

25 Leu Arg Tyr Thr Gly Glu Gly Arg Glu Asp Trp Lys Val Leu Glu Pro
 420 425 430

Ser Leu Gly Ala Arg Gly Gly Thr Leu Glu Leu Arg Pro Arg Ala Arg
 435 440 445

30 Tyr Ser Leu Gln Leu Arg Ala Arg Leu Asn Gly Pro Thr Tyr Gln Gly
 450 455 460

35 Pro Trp Ser Ala Trp Ser Pro Pro Ala Arg Val Ser Thr Gly Ser Glu
 465 470 475 480

Thr Ala Trp Ile Thr Leu Val Thr Ala Leu Leu Leu Val Leu Ser Leu
 485 490 495

40 Ser Ala Leu Leu Gly Leu Leu Leu Leu Lys Trp Gln Phe Pro Ala His
 500 505 510

Tyr Arg Arg Leu Arg His Ala Leu Trp Pro Ser Leu Pro Asp Leu His
 515 520 525

45 Arg Val Leu Gly Gln Tyr Leu Arg Asp Thr Ala Ala Leu Ser Pro Ser
 530 535 540

50 Lys Ala Thr Val Thr Asp Ser Cys Glu Glu Val Glu Pro Ser Leu Leu
 545 550 555 560

Glu Ile Leu Pro Lys Ser Ser Glu Ser Thr Pro Leu Pro Leu Cys Pro
 565 570 575

55 Ser Gln Pro Gln Met Asp Tyr Arg Gly Leu Gln Pro Cys Leu Arg Thr
 580 585 590

- 37 -

Met Pro Leu Ser Val Cys Pro Pro Met Ala Glu Thr Gly Ser Cys Cys
595 600 605

5 Thr Thr His Ile Ala Asn His Ser Tyr Leu Pro Leu Ser Tyr Trp Gln
610 615 620

Gln Pro
625

10

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
15 (A) LENGTH: 1908 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

20 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
25 (A) NAME/KEY: CDS
(B) LOCATION: 1..1908

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

30	ATG CCC TCC TGG GCC CTC TTC ATG GTC ACC TCC TGC CTC CTC CTG GCC	48
	Met Pro Ser Trp Ala Leu Phe Met Val Thr Ser Cys Leu Leu Leu Ala	
	1 5 10 15	
35	CCT CAA AAC CTG GCC CAA GTC AGC AGC CAA GAT GTC TCC TTG CTG GCA	96
	Pro Gln Asn Leu Ala Gln Val Ser Ser Gln Asp Val Ser Leu Leu Ala	
	20 25 30	
40	TCA GAC TCA GAG CCC CTG AAG TGT TTC TCC CGA ACA TTT GAG GAC CTC	144
	Ser Asp Ser Glu Pro Leu Lys Cys Phe Ser Arg Thr Phe Glu Asp Leu	
	35 40 45	
45	ACT TGC TTC TGG GAT GAG GAA GAG GCA GCG CCC AGT GGG ACA TAC CAG	192
	Thr Cys Phe Trp Asp Glu Glu Glu Ala Ala Pro Ser Gly Thr Tyr Gln	
	50 55 60	
50	CTG CTG TAT GCC TAC CCG CGG GAG AAG CCC CGT GCT TGC CCC CTG AGT	240
	Leu Leu Tyr Ala Tyr Pro Arg Glu Lys Pro Arg Ala Cys Pro Leu Ser	
	65 70 75 80	
55	TCC CAG AGC ATG CCC CAC TTT GGA ACC CGA TAC GTG TGC CAG TTT CCA	288
	Ser Gln Ser Met Pro His Phe Gly Thr Arg Tyr Val Cys Gln Phe Pro	
	85 90 95	
55	GAC CAG GAG GAA GTG CGT CTC TTC TTT CCG CTG CAC CTC TGG GTG AAG	336
	Asp Gln Glu Glu Val Arg Leu Phe Phe Pro Leu His Leu Trp Val Lys	
	100 105 110	

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	AAT GTG TTC CTA AAC CAG ACT CGG ACT CAG CGA GTC CTC TTT GTG GAC	384
	Asn Val Phe Leu Asn Gln Thr Arg Thr Gln Arg Val Leu Phe Val Asp	
	115 120 125	
5	AGT GTA GGC CTG CCG GCT CCC CCC AGT ATC ATC AAG GCC ATG GGT GGG	432
	Ser Val Gly Leu Pro Ala Pro Pro Ser Ile Ile Lys Ala Met Gly Gly	
	130 135 140	
10	AGC CAG CCA GGG GAA CTT CAG ATC AGC TGG GAG GAG CCA GCT CCA GAA	480
	Ser Gln Pro Gly Glu Leu Gln Ile Ser Trp Glu Glu Pro Ala Pro Glu	
	145 150 155 160	
15	ATC AGT GAT TTC CTG AGG TAC GAA CTC CGC TAT GGC CCC AGA GAT CCC	528
	Ile Ser Asp Phe Leu Arg Tyr Glu Leu Arg Tyr Gly Pro Arg Asp Pro	
	165 170 175	
20	AAG AAC TCC ACT GGT CCC ACG GTC ATA CAG CTG ATT GCC ACA GAA ACC	576
	Lys Asn Ser Thr Gly Pro Thr Val Ile Gln Leu Ile Ala Thr Glu Thr	
	180 185 190	
25	TGC TGC CCT GCT CTG CAG AGG CCT CAC TCA GCC TCT GCT CTG GAC CAG	624
	Cys Cys Pro Ala Leu Gln Arg Pro His Ser Ala Ser Ala Leu Asp Gln	
	195 200 205	
30	TCT CCA TGT GCT CAG CCC ACA ATG CCC TGG CAA GAT GGA CCA AAG CAG	672
	Ser Pro Cys Ala Gln Pro Thr Met Pro Trp Gln Asp Gly Pro Lys Gln	
	210 215 220	
35	ACC TCC CCA AGT AGA GAA GCT TCA GCT CTG ACA GCA GAG GGT GGA AGC	720
	Thr Ser Pro Ser Arg Glu Ala Ser Ala Leu Thr Ala Glu Gly Gly Ser	
	225 230 235 240	
40	TGC CTC ATC TCA GGA CTC CAG CCT GGC AAC TCC TAC TGG CTG CAG CTG	768
	Cys Leu Ile Ser Gly Leu Gln Pro Gly Asn Ser Tyr Trp Leu Gln Leu	
	245 250 255	
45	CGC AGC GAA CCT GAT GGG ATC TCC CTC GGT GGC TCC TGG GGA TCC TGG	816
	Arg Ser Glu Pro Asp Gly Ile Ser Leu Gly Gly Ser Trp Gly Ser Trp	
	260 265 270	
50	TCC CTC CCT GTG ACT GTG GAC CTG CCT GGA GAT GCA GTG GCA CTT GGA	864
	Ser Leu Pro Val Thr Val Asp Leu Pro Gly Asp Ala Val Ala Leu Gly	
	275 280 285	
55	CTG CAA TGC TTT ACC TTG GAC CTG AAG AAT GTT ACC TGT CAA TGG CAG	912
	Leu Gln Cys Phe Thr Leu Asp Leu Lys Asn Val Thr Cys Gln Trp Gln	
	290 295 300	
60	CAA CAG GAC CAT GCT AGC TCC CAA GGC TTC TTC TAC CAC AGC AGG GCA	960
	Gln Gln Asp His Ala Ser Ser Gln Gly Phe Phe Tyr His Ser Arg Ala	
	305 310 315 320	
65	CGG TGC TGC CCC AGA GAC AGG TAC CCC ATC TGG GAG AAC TGC GAA GAG	1008
	Arg Cys Cys Pro Arg Asp Arg Tyr Pro Ile Trp Glu Asn Cys Glu Glu	
	325 330 335	

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	GAA GAG AAA ACA AAT CCA GGA CTA CAG ACC CCA CAG TTC TCT CGC TGC	1056
	Glu Glu Lys Thr Asn Pro Gly Leu Gln Thr Pro Gln Phe Ser Arg Cys	
	340 345 350	
5	CAC TTC AAG TCA CGA AAT GAC AGC ATT ATT CAC ATC CTT GTG GAG GTG	1104
	His Phe Lys Ser Arg Asn Asp Ser Ile Ile His Ile Leu Val Glu Val	
	355 360 365	
10	ACC ACA GCC CCG GGT ACT GTT CAC AGC TAC CTG GGC TCC CCT TTC TGG	1152
	Thr Thr Ala Pro Gly Thr Val His Ser Tyr Leu Gly Ser Pro Phe Trp	
	370 375 380	
15	ATC CAC CAG GCT GTG CGC CTC CCC ACC CCA AAC TTG CAC TGG AGG GAG	1200
	Ile His Gln Ala Val Leu Pro Thr Pro Asn Leu His Trp Arg Glu	
	385 390 395 400	
20	ATC TCC AGT GGG CAT CTG GAA TTG GAG TGG CAG CAC CCA TCG TCC TGG	1248
	Ile Ser Ser Gly His Leu Glu Leu Glu Trp Gln His Pro Ser Ser Trp	
	405 410 415	
25	GCA GCC CAA GAG ACC TGT TAT CAA CTC CGA TAC ACA GGA GAA GGC CAT	1296
	Ala Ala Gln Glu Thr Cys Tyr Gln Leu Arg Tyr Thr Gly Glu Gly His	
	420 425 430	
30	CAG GAC TGG AAG GTG CTG GAG CCG CCT CTC GGC GCC CGA GGA GGG ACC	1344
	Gln Asp Trp Lys Val Leu Glu Pro Pro Leu Gly Ala Arg Gly Gly Thr	
	435 440 445	
35	CTG GAG CTG CGC CCG CGA TCT CGC TAC CGT TTA CAG CTG CGC GCC AGG	1392
	Leu Glu Leu Arg Pro Arg Ser Arg Tyr Arg Leu Gln Leu Arg Ala Arg	
	450 455 460	
40	CTC AAC GGC CCC ACC TAC CAA GGT CCC TGG AGC TCG TGG TCG GAC CCA	1440
	Leu Asn Gly Pro Thr Tyr Gln Gly Pro Trp Ser Ser Trp Ser Asp Pro	
	465 470 475 480	
45	ACT AGG GTG GAG ACC GCC ACC GAG ACC GCC TGG ATC TCC TTG GTG ACC	1488
	Thr Arg Val Glu Thr Ala Thr Glu Thr Ala Trp Ile Ser Leu Val Thr	
	485 490 495	
50	GCT CTG CAT CTA GTG CTG GGC CTC AGC GCC GTC CTG GGC CTG CTG CTG	1536
	Ala Leu His Leu Val Leu Gly Leu Ser Ala Val Leu Gly Leu Leu Leu	
	500 505 510	
55	CTG AGG TGG CAG TTT CCT GCA CAC TAC AGG AGA CTG AGG CAT GCC CTG	1584
	Leu Arg Trp Gln Phe Pro Ala His Tyr Arg Arg Leu Arg His Ala Leu	
	515 520 525	
60	TGG CCC TCA CTT CCA GAC CTG CAC CGG GTC CTA GGC CAG TAC CTT AGG	1632
	Trp Pro Ser Leu Pro Asp Leu His Arg Val Leu Gly Gln Tyr Leu Arg	
	530 535 540	
65	GAC ACT GCA GCC CTG AGC CCG CCC AAG GCC ACA GTC TCA GAT ACC TGT	1680
	Asp Thr Ala Ala Leu Ser Pro Pro Lys Ala Thr Val Ser Asp Thr Cys	
	545 550 555 560	

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	GAA	GAA	GTG	GAA	CCC	AGC	CTC	CTT	GAA	ATC	CTC	CCC	AAG	TCC	TCA	GAG	1728
	Glu	Glu	Val	Glu	Pro	Ser	Leu	Leu	Glu	Ile	Leu	Pro	Lys	Ser	Ser	Glu	
					565					570						575	
5	AGG	ACT	CCT	TTG	CCC	CTG	TGT	TCC	TCC	CAG	GCC	CAG	ATG	GAC	TAC	CGA	1776
	Arg	Thr	Pro	Leu	Pro	Leu	Cys	Ser	Ser	Gln	Ala	Gln	Met	Asp	Tyr	Arg	
					580					585						590	
10	AGA	TTG	CAG	CCT	TCT	TGC	CTG	GGG	ACC	ATG	CCC	CTG	TCT	GTG	TGC	CCA	1824
	Arg	Leu	Gln	Pro	Ser	Cys	Leu	Gly	Thr	Met	Pro	Leu	Ser	Val	Cys	Pro	
					595					600						605	
15	CCC	ATG	GCT	GAG	TCA	GGG	TCC	TGC	TGT	ACC	ACC	CAC	ATT	GCC	AAC	CAT	1872
	Pro	Met	Ala	Glu	Ser	Gly	Ser	Cys	Cys	Thr	Thr	His	Ile	Ala	Asn	His	
					610					615						620	
20	TCC	TAC	CTA	CCA	CTA	AGC	TAT	TGG	CAG	CAG	CCT	TG					1908
	Ser	Tyr	Leu	Pro	Leu	Ser	Tyr	Trp	Gln	Gln	Pro						
	625					630					635						

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 635 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

	Met	Pro	Ser	Trp	Ala	Leu	Phe	Met	Val	Thr	Ser	Cys	Leu	Leu	Leu	Ala
	1					5										15
35	Pro	Gln	Asn	Leu	Ala	Gln	Val	Ser	Ser	Gln	Asp	Val	Ser	Leu	Leu	Ala
				20					25						30	
	Ser	Asp	Ser	Glu	Pro	Leu	Lys	Cys	Phe	Ser	Arg	Thr	Phe	Glu	Asp	Leu
40			35					40					45			
	Thr	Cys	Phe	Trp	Asp	Glu	Glu	Glu	Ala	Ala	Pro	Ser	Gly	Thr	Tyr	Gln
		50					55					60				
45	Leu	Leu	Tyr	Ala	Tyr	Pro	Arg	Glu	Lys	Pro	Arg	Ala	Cys	Pro	Leu	Ser
	65					70					75					80
	Ser	Gln	Ser	Met	Pro	His	Phe	Gly	Thr	Arg	Tyr	Val	Cys	Gln	Phe	Pro
					85					90					95	
50	Asp	Gln	Glu	Glu	Val	Arg	Leu	Phe	Phe	Pro	Leu	His	Leu	Trp	Val	Lys
				100					105					110		
	Asn	Val	Phe	Leu	Asn	Gln	Thr	Arg	Thr	Gln	Arg	Val	Leu	Phe	Val	Asp
55			115					120					125			
	Ser	Val	Gly	Leu	Pro	Ala	Pro	Pro	Ser	Ile	Ile	Lys	Ala	Met	Gly	Gly
	130						135					140				

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Ser Gln Pro Gly Glu Leu Gln Ile Ser Trp Glu Glu Pro Ala Pro Glu
 145 150 155 160
 5 Ile Ser Asp Phe Leu Arg Tyr Glu Leu Arg Tyr Gly Pro Arg Asp Pro
 165 170 175
 Lys Asn Ser Thr Gly Pro Thr Val Ile Gln Leu Ile Ala Thr Glu Thr
 180 185 190
 10 Cys Cys Pro Ala Leu Gln Arg Pro His Ser Ala Ser Ala Leu Asp Gln
 195 200 205
 Ser Pro Cys Ala Gln Pro Thr Met Pro Trp Gln Asp Gly Pro Lys Gln
 210 215 220
 15 Thr Ser Pro Ser Arg Glu Ala Ser Ala Leu Thr Ala Glu Gly Gly Ser
 225 230 235 240
 Cys Leu Ile Ser Gly Leu Gln Pro Gly Asn Ser Tyr Trp Leu Gln Leu
 245 250 255
 20 Arg Ser Glu Pro Asp Gly Ile Ser Leu Gly Gly Ser Trp Gly Ser Trp
 260 265 270
 25 Ser Leu Pro Val Thr Val Asp Leu Pro Gly Asp Ala Val Ala Leu Gly
 275 280 285
 Leu Gln Cys Phe Thr Leu Asp Leu Lys Asn Val Thr Cys Gln Trp Gln
 290 295 300
 30 Gln Gln Asp His Ala Ser Ser Gln Gly Phe Phe Tyr His Ser Arg Ala
 305 310 315 320
 Arg Cys Cys Pro Arg Asp Arg Tyr Pro Ile Trp Glu Asn Cys Glu Glu
 325 330 335
 35 Glu Glu Lys Thr Asn Pro Gly Leu Gln Thr Pro Gln Phe Ser Arg Cys
 340 345 350
 40 His Phe Lys Ser Arg Asn Asp Ser Ile Ile His Ile Leu Val Glu Val
 355 360 365
 Thr Thr Ala Pro Gly Thr Val His Ser Tyr Leu Gly Ser Pro Phe Trp
 370 375 380
 45 Ile His Gln Ala Val Ala Leu Pro Thr Pro Asn Leu His Trp Arg Glu
 385 390 395 400
 Ile Ser Ser Gly His Leu Glu Leu Glu Trp Gln His Pro Ser Ser Trp
 405 410 415
 Ala Ala Gln Glu Thr Cys Tyr Gln Leu Arg Tyr Thr Gly Glu Gly His
 420 425 430
 55 Gln Asp Trp Lys Val Leu Glu Pro Pro Leu Gly Ala Arg Gly Gly Thr
 435 440 445
 Leu Glu Leu Arg Pro Arg Ser Arg Tyr Arg Leu Gln Leu Arg Ala Arg

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	450	455	460
	Leu Asn Gly Pro Thr Tyr Gln Gly Pro Trp Ser Ser Trp Ser Asp Pro		
	465	470	475 480
5	Thr Arg Val Glu Thr Ala Thr Glu Thr Ala Trp Ile Ser Leu Val Thr		
		485 490	495
10	Ala Leu His Leu Val Leu Gly Leu Ser Ala Val Leu Gly Leu Leu Leu		
		500 505	510
	Leu Arg Trp Gln Phe Pro Ala His Tyr Arg Arg Leu Arg His Ala Leu		
		515 520	525
15	Trp Pro Ser Leu Pro Asp Leu His Arg Val Leu Gly Gln Tyr Leu Arg		
		530 535	540
	Asp Thr Ala Ala Leu Ser Pro Pro Lys Ala Thr Val Ser Asp Thr Cys		
		545 550	555 560
20	Glu Glu Val Glu Pro Ser Leu Leu Glu Ile Leu Pro Lys Ser Ser Glu		
		565 570	575
25	Arg Thr Pro Leu Pro Leu Cys Ser Ser Gln Ala Gln Met Asp Tyr Arg		
		580 585	590
	Arg Leu Gln Pro Ser Cys Leu Gly Thr Met Pro Leu Ser Val Cys Pro		
		595 600	605
30	Pro Met Ala Glu Ser Gly Ser Cys Cys Thr Thr His Ile Ala Asn His		
		610 615	620
	Ser Tyr Leu Pro Leu Ser Tyr Trp Gln Gln Pro		
		625 630	635

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

50 TACAAGCTTG CCGTCATCAT GCCCTCTTGG GCCCTC 36

55

(2) INFORMATION FOR SEQ ID NO:6:

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(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 33 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ACTTCTAGAC TATCAAGCAG TCTCGGAGCT GGA 33

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1523 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: unknown
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 69..1514

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGATCCTCTA GAGCGGCCGC TAAGGCAGGC ACACAGTGCC GGAGAAGATG CCCTCTTGGG 60

CCCTCTTC ATG GTC ACC TCC TGC CTC CTC TTG GCC CTT CCA AAC CAG GCA 110
 Met Val Thr Ser Cys Leu Leu Leu Ala Leu Pro Asn Gln Ala
 1 5 10

CAA GTC ACC AGC CAA GAT GTC TTC TTG CTG GCC TTG GGC ACA GAG CCC 158
 Gln Val Thr Ser Gln Asp Val Phe Leu Leu Ala Leu Gly Thr Glu Pro
 15 20 25 30

CTG AAC TGC TTC TCC CAA ACA TTT GAG GAC CTC ACC TGC TTC TGG GAT 206
 Leu Asn Cys Phe Ser Gln Thr Phe Glu Asp Leu Thr Cys Phe Trp Asp
 35 40 45

GAG GAA GAG GCA GCA CCC AGT GGG ACA TAC CAG CTG CTG TAT GCC TAC 254
 Glu Glu Glu Ala Ala Pro Ser Gly Thr Tyr Gln Leu Leu Tyr Ala Tyr
 50 55 60

CGA GGA GAG AAG CCC CGT GCA TGC CCC CTG TAT TCC CAG AGT GTG CCC 302
 Arg Gly Glu Lys Pro Arg Ala Cys Pro Leu Tyr Ser Gln Ser Val Pro
 65 70 75

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	ACC TTT GGA ACC CGG TAT GTG TGC CAG TTT CCA GCC CAG GAT GAA GTG	350
	Thr Phe Gly Thr Arg Tyr Val Cys Gln Phe Pro Ala Gln Asp Glu Val	
	80 85 90	
5	CGC CTC TTC TTT CCG CTG CAC CTC TGG GTG AAG AAT GTG TCC CTC AAC	398
	Arg Leu Phe Phe Pro Leu His Leu Trp Val Lys Asn Val Ser Leu Asn	
	95 100 105 110	
10	CAG ACT TTG ATC CAG CGG GTG CTG TTT GTG GAT AGT GTG GGC CTG CCA	446
	Gln Thr Leu Ile Gln Arg Val Leu Phe Val Asp Ser Val Gly Leu Pro	
	115 120 125	
15	GCT CCC CCC AGG GTC ATC AAG GCC AGG GGT GGG AGC CAA CCA GGG GAA	494
	Ala Pro Pro Arg Val Ile Lys Ala Arg Gly Gly Ser Gln Pro Gly Glu	
	130 135 140	
20	CTT CAG ATC CAC TGG GAG GCC CCT GCT CCT GAA ATC AGT GAC TTC CTG	542
	Leu Gln Ile His Trp Glu Ala Pro Ala Pro Glu Ile Ser Asp Phe Leu	
	145 150 155	
25	AGG CAT GAA CTC CGC TAT GGC CCC ACG GAT TCC AGC AAC GCC ACT GCC	590
	Arg His Glu Leu Arg Tyr Gly Pro Thr Asp Ser Ser Asn Ala Thr Ala	
	160 165 170	
30	CCC TCC GTC ATT CAG CTG CTC TCC ACA GAA ACC TGC TGC CCC ACT TTG	638
	Pro Ser Val Ile Gln Leu Leu Ser Thr Glu Thr Cys Cys Pro Thr Leu	
	175 180 185 190	
35	TGG ATG CCG AAC CCA GTC CCT GTT CTT GAC CAG CCT CCG TGT GTT CAT	686
	Trp Met Pro Asn Pro Val Pro Val Leu Asp Gln Pro Pro Cys Val His	
	195 200 205	
40	CCG ACA GCA TCC CAA CCG CAT GGA CCA GTG AGG ACC TCC CCA GCT GGA	734
	Pro Thr Ala Ser Gln Pro His Gly Pro Val Arg Thr Ser Pro Ala Gly	
	210 215 220	
45	GAA GCT CCA TTT CTG ACA GTG AAG GGT GGA AGC TGT CTC GTC TCA GGC	782
	Glu Ala Pro Phe Leu Thr Val Lys Gly Gly Ser Cys Leu Val Ser Gly	
	225 230 235	
50	CTC CAG GCT AGC AAA TCC TAC TGG CTC CAG CTA CGC AGC CAA CCC GAC	830
	Leu Gln Ala Ser Lys Ser Tyr Trp Leu Gln Leu Arg Ser Gln Pro Asp	
	240 245 250	
55	GGG GTC TCT CTT CGT GGC TCC TGG GGA CCC TGG TCC TTC CCT GTG ACT	878
	Gly Val Ser Leu Arg Gly Ser Trp Gly Pro Trp Ser Phe Pro Val Thr	
	255 260 265 270	
60	GTG GAT CTT CCA GGA GAT GCA GTG ACA ATT GGA CTT CAG TGC TTT ACC	926
	Val Asp Leu Pro Gly Asp Ala Val Thr Ile Gly Leu Gln Cys Phe Thr	
	275 280 285	
65	TTG GAT CTG AAG ATG GTC ACC TGC CAG TGG CAG CAA CAA GAC CGC ACT	974
	Leu Asp Leu Lys Met Val Thr Cys Gln Trp Gln Gln Gln Asp Arg Thr	
	290 295 300	

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	AGC TCC CAA GGC TTC TTC CGT CAC AGC AGG ACG AGG TGC TGC CCC ACA	1022
	Ser Ser Gln Gly Phe Phe Arg His Ser Arg Thr Arg Cys Cys Pro Thr	
	305 310 315	
5	GAC AGG GAC CCC ACC TGG GAG AAA TGT GAA GAC GAG GAA CCG CGT CCA	1070
	Asp Arg Asp Pro Thr Trp Glu Lys Cys Glu Glu Glu Glu Pro Arg Pro	
	320 325 330	
10	GGA TCA CAG CCC GCT CTC GTC TCC CGC TGC CAC TTC AAG TCA CGA AAT	1118
	Gly Ser Gln Pro Ala Leu Val Ser Arg Cys His Phe Lys Ser Arg Asn	
	335 340 345 350	
15	GAC AGT GTT ATT CAC ATC CTT GTA GAG GTG ACC ACA GCG CAA GGT GCC	1166
	Asp Ser Val Ile His Ile Leu Val Glu Val Thr Thr Ala Gln Gly Ala	
	355 360 365	
20	GTT CAC AGC TAC CTG GGC TCC CCT TTT TGG ATC CAC CAG GCT GTG CTC	1214
	Val His Ser Tyr Leu Gly Ser Pro Phe Trp Ile His Gln Ala Val Leu	
	370 375 380	
25	CTT CCC ACC CCG AGC CTG CAC TGG AGG GAG GTC TCA AGT GGA AGG CTG	1262
	Leu Pro Thr Pro Ser Leu His Trp Arg Glu Val Ser Ser Gly Arg Leu	
	385 390 395	
30	GAG TTG GAG TGG CAG CAC CAG TCA TCT TGG GCA GCT CAA GAG ACC TGC	1310
	Glu Leu Glu Trp Gln His Gln Ser Ser Trp Ala Ala Gln Glu Thr Cys	
	400 405 410	
35	TAC CAG CTC CGG TAC ACG GGA GAA GGC CGT GAG GAC TGG AAG GTG CTG	1358
	Tyr Gln Leu Arg Tyr Thr Gly Glu Gly Arg Glu Asp Trp Lys Val Leu	
	415 420 425 430	
40	GAG CCA TCT CTC GGT GCC CGG GGA GGG ACC CTA GAG CTG CGC CCC CGA	1406
	Glu Pro Ser Leu Gly Ala Arg Gly Gly Thr Leu Glu Leu Arg Pro Arg	
	435 440 445	
45	GCT CGC TAC AGC TTG CAG CTG CGT GCC AGG CTC AAC GGC CCC ACC TAC	1454
	Ala Arg Tyr Ser Leu Gln Leu Arg Ala Arg Leu Asn Gly Pro Thr Tyr	
	450 455 460	
50	CAA GGT CCC TGG AGC GCC TGG TCT CCC CCA GCT AGG GTG TCC ACG GGC	1502
	Gln Gly Pro Trp Ser Ala Trp Ser Pro Pro Ala Arg Val Ser Thr Gly	
	465 470 475	
55	TCC GAG ACT GCT TGAGTCGAC	1523
	Ser Glu Thr Ala	
	480	
50	(2) INFORMATION FOR SEQ ID NO:8:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 482 amino acids	
	(B) TYPE: amino acid	
	(D) TOPOLOGY: linear	
55	(ii) MOLECULE TYPE: protein	

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

5	Met	Val	Thr	Ser	Cys	Leu	Leu	Leu	Ala	Leu	Pro	Asn	Gln	Ala	Gln	Val	1	5	10	15
	Thr	Ser	Gln	Asp	Val	Phe	Leu	Leu	Ala	Leu	Gly	Thr	Glu	Pro	Leu	Asn	20	25	30	
10	Cys	Phe	Ser	Gln	Thr	Phe	Glu	Asp	Leu	Thr	Cys	Phe	Trp	Asp	Glu	Glu	35	40	45	
	Glu	Ala	Ala	Pro	Ser	Gly	Thr	Tyr	Gln	Leu	Leu	Tyr	Ala	Tyr	Arg	Gly	50	55	60	
15	Glu	Lys	Pro	Arg	Ala	Cys	Pro	Leu	Tyr	Ser	Gln	Ser	Val	Pro	Thr	Phe	65	70	75	80
	Gly	Thr	Arg	Tyr	Val	Cys	Gln	Phe	Pro	Ala	Gln	Asp	Glu	Val	Arg	Leu	85	90	95	
20	Ph	Phe	Pro	Leu	His	Leu	Trp	Val	Lys	Asn	Val	Ser	Leu	Asn	Gln	Thr	100	105	110	
	Leu	Ile	Gln	Arg	Val	Leu	Phe	Val	Asp	Ser	Val	Gly	Leu	Pro	Ala	Pro	115	120	125	
25	Pro	Arg	Val	Ile	Lys	Ala	Arg	Gly	Gly	Ser	Gln	Pro	Gly	Glu	Leu	Gln	130	135	140	
30	Ile	His	Trp	Glu	Ala	Pro	Ala	Pro	Glu	Ile	Ser	Asp	Phe	Leu	Arg	His	145	150	155	160
	Glu	Leu	Arg	Tyr	Gly	Pro	Thr	Asp	Ser	Ser	Asn	Ala	Thr	Ala	Pro	Ser	165	170	175	
35	Val	Ile	Gln	Leu	Leu	Ser	Thr	Glu	Thr	Cys	Cys	Pro	Thr	Leu	Trp	Met	180	185	190	
	Pro	Asn	Pro	Val	Pro	Val	Leu	Asp	Gln	Pro	Pro	Cys	Val	His	Pro	Thr	195	200	205	
40	Ala	Ser	Gln	Pro	His	Gly	Pro	Val	Arg	Thr	Ser	Pro	Ala	Gly	Glu	Ala	210	215	220	
45	Pro	Phe	Leu	Thr	Val	Lys	Gly	Gly	Ser	Cys	Leu	Val	Ser	Gly	Leu	Gln	225	230	235	240
	Ala	Ser	Lys	Ser	Tyr	Trp	Leu	Gln	Leu	Arg	Ser	Gln	Pro	Asp	Gly	Val	245	250	255	
50	Ser	Leu	Arg	Gly	Ser	Trp	Gly	Pro	Trp	Ser	Phe	Pro	Val	Thr	Val	Asp	260	265	270	
55	Leu	Pro	Gly	Asp	Ala	Val	Thr	Ile	Gly	Leu	Gln	Cys	Phe	Thr	Leu	Asp	275	280	285	
	Leu	Lys	Met	Val	Thr	Cys	Gln	Trp	Gln	Gln	Gln	Asp	Arg	Thr	Ser	Ser				

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	290	295	300
5	Gln Gly Phe Phe Arg His Ser Arg Thr Arg Cys Cys Pro Thr Asp Arg 305 310 315 320		
	Asp Pro Thr Trp Glu Lys Cys Glu Glu Glu Glu Pro Arg Pro Gly Ser 325 330 335		
10	Gln Pro Ala Leu Val Ser Arg Cys His Phe Lys Ser Arg Asn Asp Ser 340 345 350		
	Val Ile His Ile Leu Val Glu Val Thr Thr Ala Gln Gly Ala Val His 355 360 365		
15	Ser Tyr Leu Gly Ser Pro Phe Trp Ile His Gln Ala Val Leu Leu Pro 370 375 380		
	Thr Pro Ser Leu His Trp Arg Glu Val Ser Ser Gly Arg Leu Glu Leu 385 390 395 400		
	Glu Trp Gln His Gln Ser Ser Trp Ala Ala Gln Glu Thr Cys Tyr Gln 405 410 415		
25	Leu Arg Tyr Thr Gly Glu Gly Arg Glu Asp Trp Lys Val Leu Glu Pro 420 425 430		
	Ser Leu Gly Ala Arg Gly Gly Thr Leu Glu Leu Arg Pro Arg Ala Arg 435 440 445		
30	Tyr Ser Leu Gln Leu Arg Ala Arg Leu Asn Gly Pro Thr Tyr Gln Gly 450 455 460		
	Pro Trp Ser Ala Trp Ser Pro Pro Ala Arg Val Ser Thr Gly Ser Glu 465 470 475 480		
35	Thr Ala		

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WHAT IS CLAIMED IS:

1. A method for increasing the number of platelets in a mammal, which comprises administering to
5 the mammal a platelet number increasing effective amount of an unbound MPL receptor.
2. A method according to Claim 1, wherein
said unbound MPL receptor is a soluble MPL receptor.
10
3. A method according to Claim 2, wherein
said soluble MPL receptor is mammalian.
4. A method according to Claim 3 wherein said
15 soluble mammalian MPL receptor is selected from the group consisting of murine and human.
5. A method according to Claim 2, wherein
said soluble MPL receptor is selected from the group
20 consisting of polypeptides having a sequence of from residue 19 (± 10 amino acids) to residue 483 (± 10 amino acids) of SEQ. ID NO. 2.
6. A method according to Claim 2, wherein
25 said soluble MPL receptor is selected from the group consisting of polypeptides having a sequence of from residue 26 (± 10 amino acids) to residue 491 (± 10 amino acids) of SEQ. ID NO. 4.
7. A method according to Claim 2, wherein
30 said soluble MPL receptor is soluble in a pharmaceutically acceptable liquid.
8. A method according to Claim 2, wherein
35 said soluble MPL receptor is soluble in a body fluid.

9. A method according to claim 6, wherein said mammal is human.

10. A method according to claim 6, wherein said mammal is suffering from a platelet deficiency.

11. A method according to claim 1, wherein said administration is carried out
5 intravenously.

12. A method according to claim 6, wherein said platelet increasing amount is from about 1 μg per kg to about 100 μg per kg per day.

13. A method for increasing the number of platelets, substantially as herein described with reference to Example 5 or Example 6.

10 DATED this 15th day of July, 1997

AMGEN INC

Attorney: IAN T. ERNST

Fellow Institute of Patent Attorneys of Australia

of SHELSTON WATERS

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Fig. 1

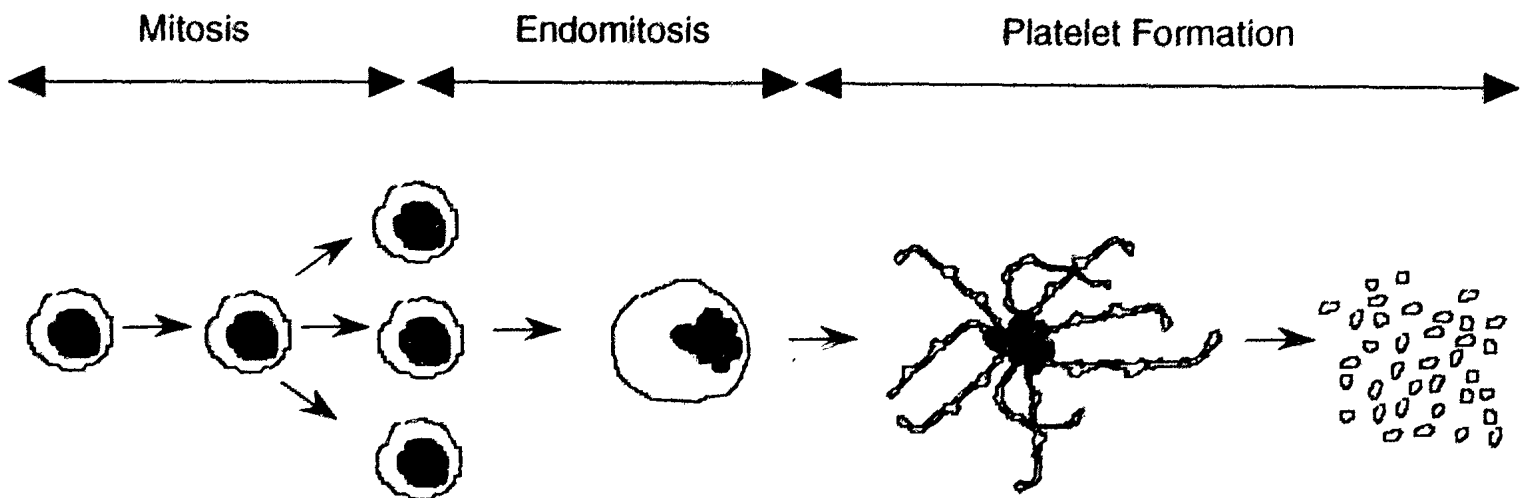


Fig. 2

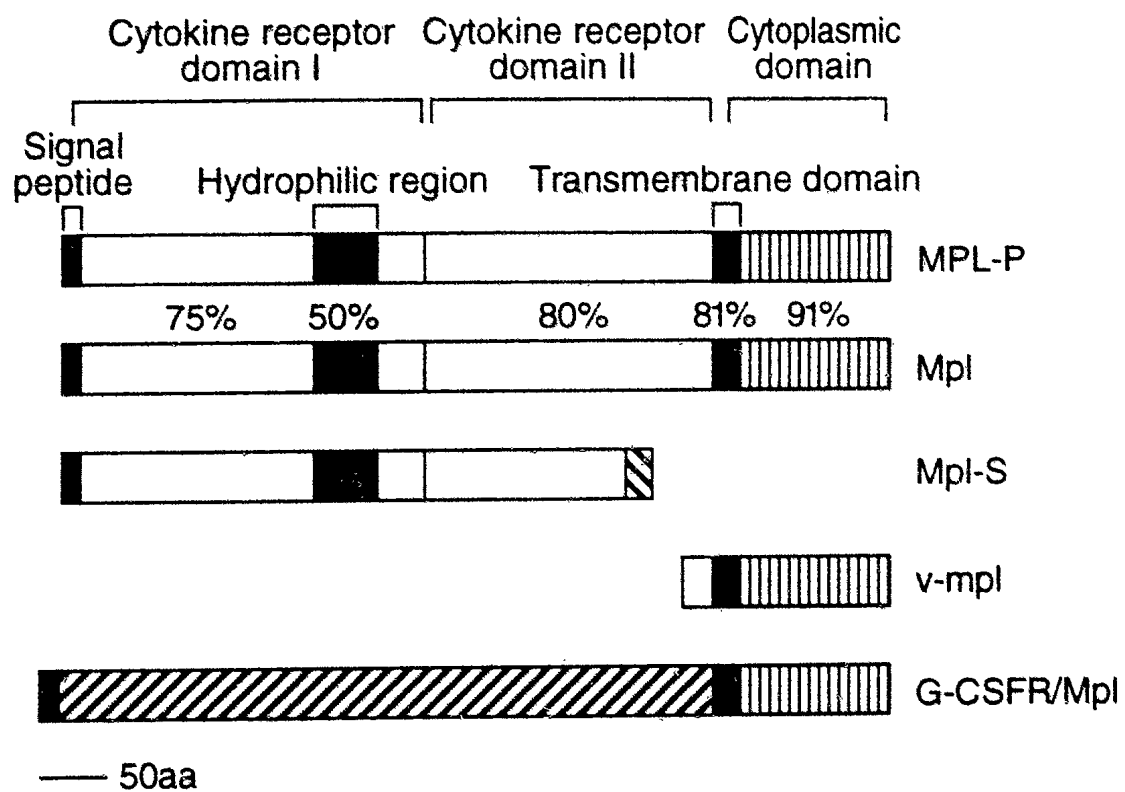


Fig. 3

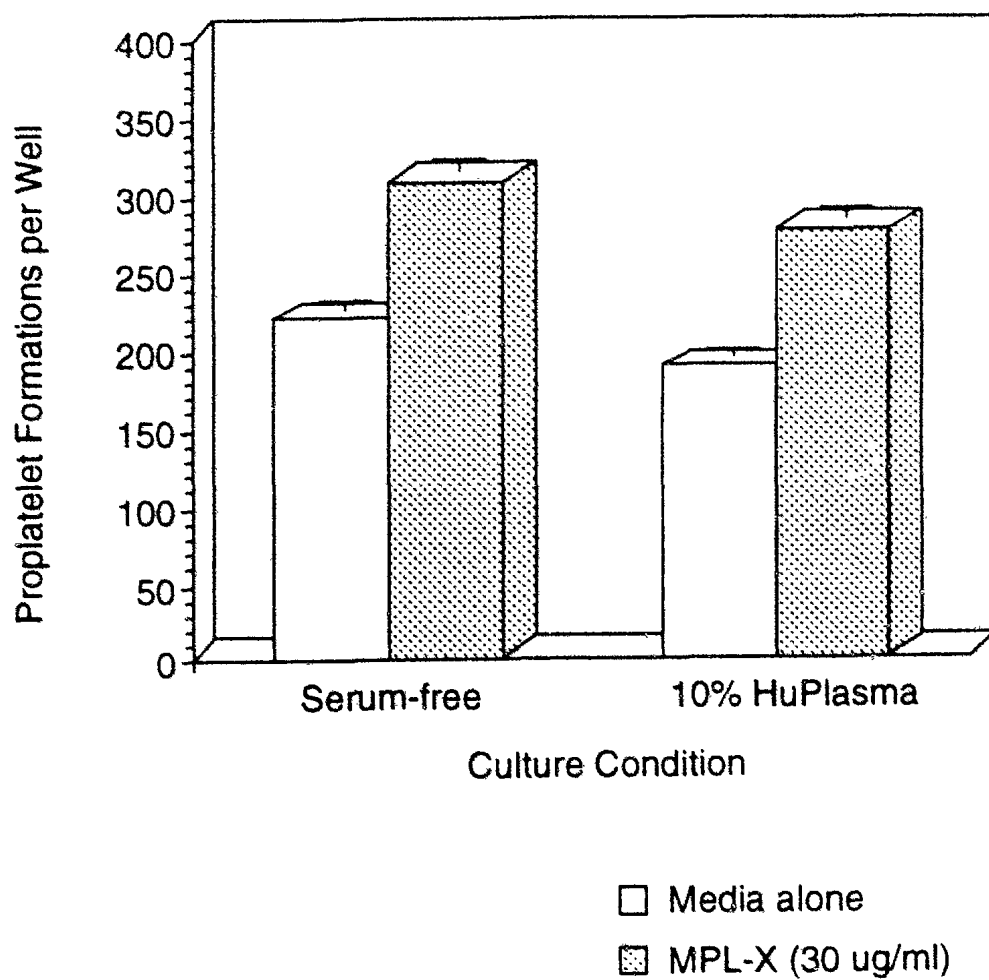


Fig. 4

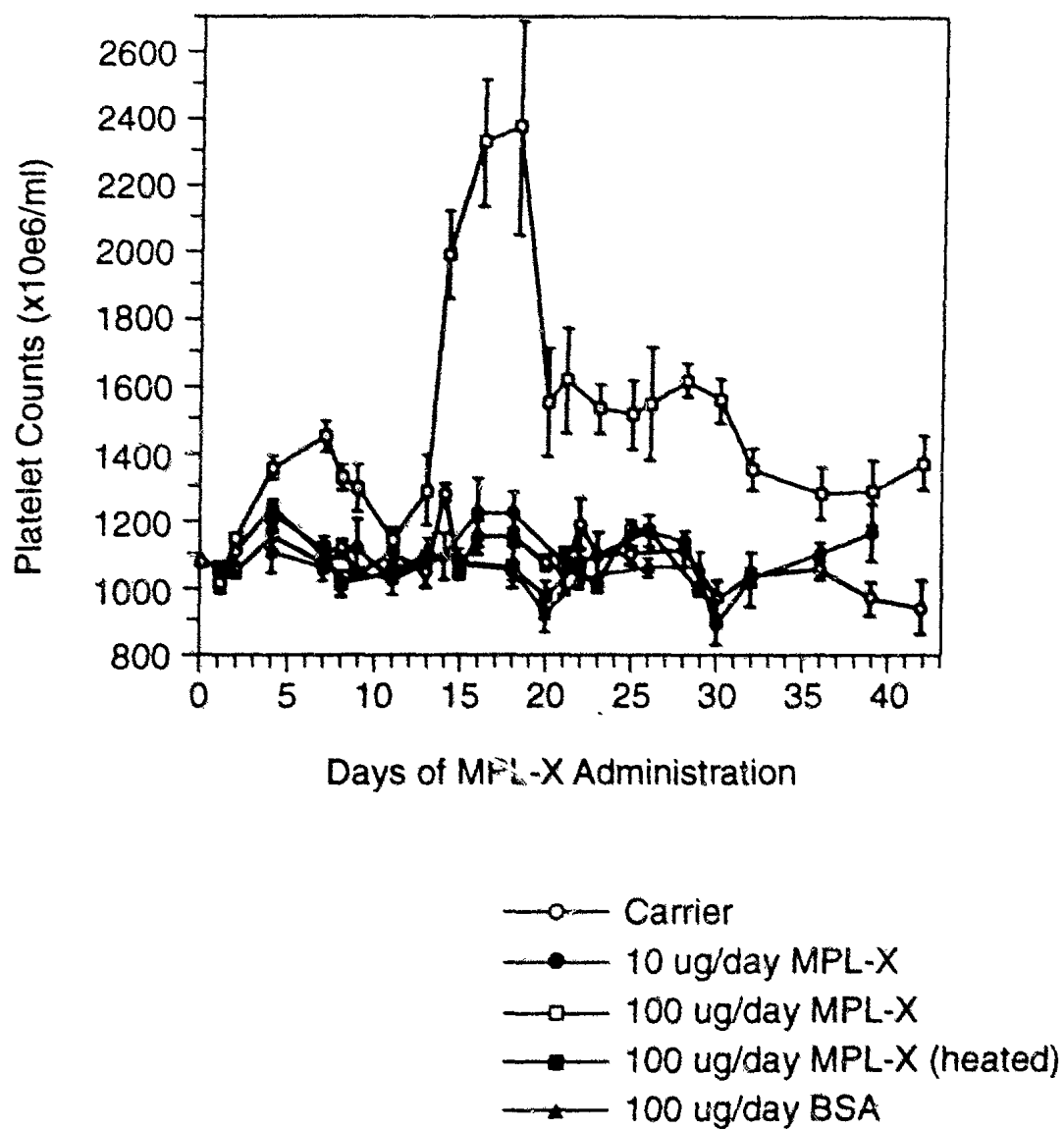


Fig. 5

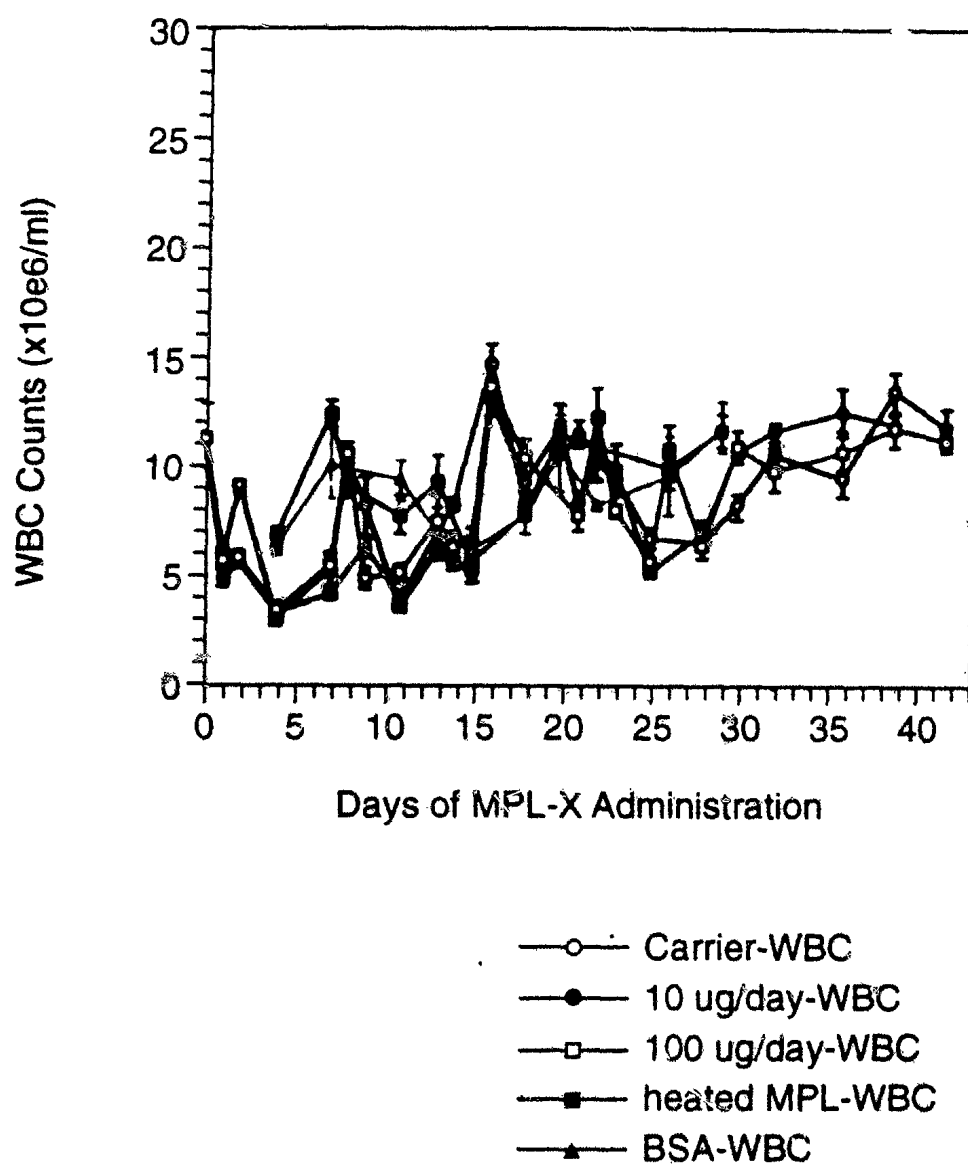
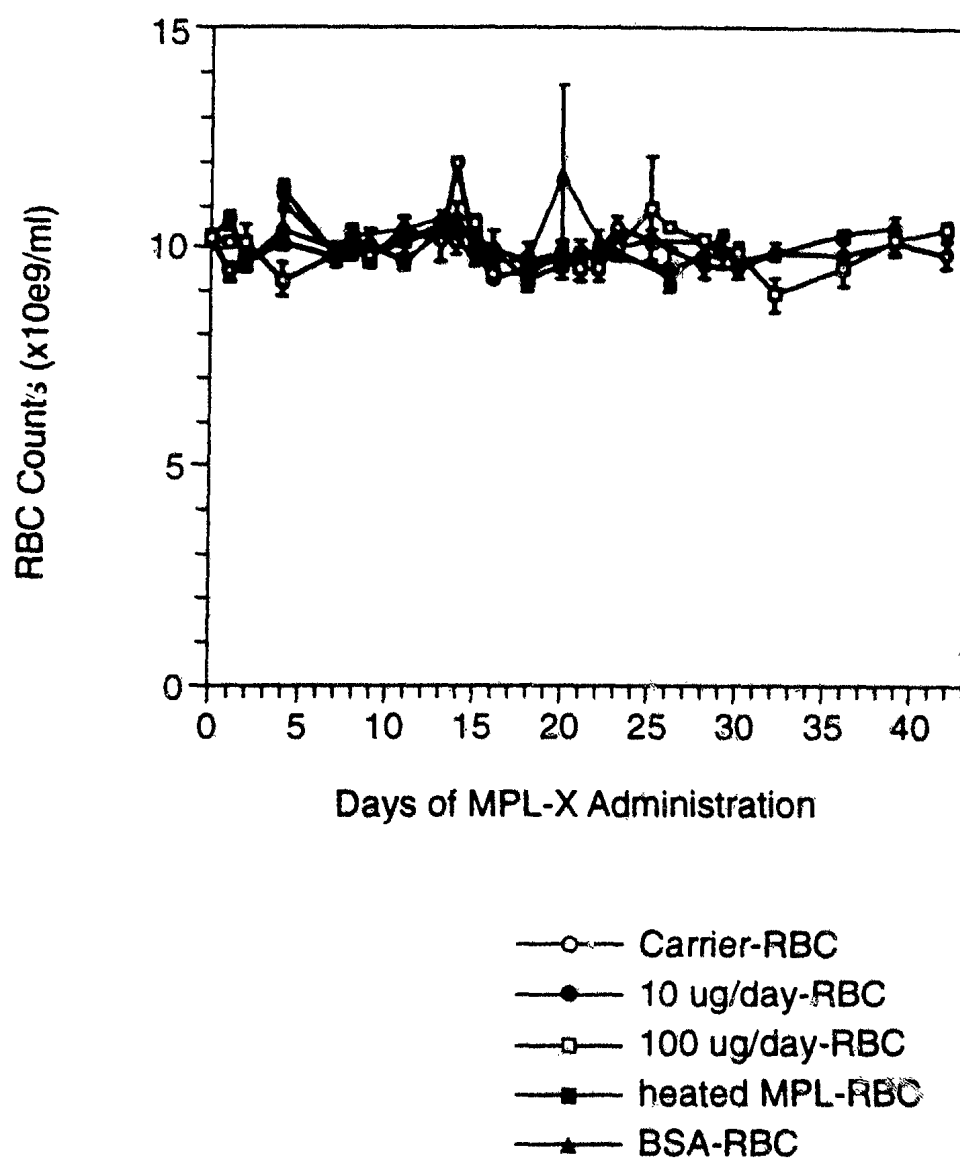


Fig. 6



INTERNATIONAL SEARCH REPORT

Inter national Application No
PCT/US 95/00670

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/71 A61K38/17

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

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 practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,92 07074 (INSERM) 30 April 1992 cited in the application ----	
A	BLOOD, vol. 82,no. 5, 1 September 1993 pages 1395-1401, METHIA,N. ET AL. 'Oligodeoxynucleotides antisense to the proto-oncogene c-mpl specifically inhibit in vitro megakaryo- cytopoiesis' cited in the application -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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- * "E" earlier document but published on or after the international filing date
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Date of the actual completion of the international search

8 June 1995

Date of mailing of the international search report

21.06.95

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INTERNATIONAL SEARCH REPORT

Internal Application No

PCT/US 95/00670

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9207074	30-04-92	JP-T- 6501915	03-03-94
