Title: A METHOD OF TREATMENT AND PROPHYLAXIS OF EVENTS, CONDITIONS AND DISEASES OF THE SYSTEMIC VASCULATURE AN IMMUNE FUNCTION TO DECREASE CARDIOVASCULAR RISK AND PATHOGENIC INFECTION

Abstract: The present invention relates generally to the treatment and prophylaxis of events, conditions and diseases of the systemic vasculature and treatment and prophylaxis of infection by pathogenic agents. Agents are contemplated which enhance levels of stem cells and/or facilitate the angiogenic or vasculogenic capability of stem cells or their precursors or early committed cells of a particular cell lineage or to mobilize stem cells to thereby promote repair and maintenance of elements and components of the systemic vasculature. A method is also provided to activate hypoxic gene programming in target tissues to induce angiogenesis and recruit progenitor cells to that site. Still further, a method to facilitate enhancement of immune function is also provided. Agents and pharmaceutical compositions including functional foods and specialized drug delivery devices useful in the treatment and prophylaxis of events, conditions and disease of the systemic vasculature and to enhance the immune system are contemplated.
A method of treatment and prophylaxis of events, conditions and diseases of the systemic vasculature and immune function to decrease cardiovascular risk and pathogenic infection

FIELD

The present invention relates generally to the treatment and prophylaxis of events, conditions and diseases of the systemic vasculature and treatment and prophylaxis of infection by pathogenic agents. Agents are contemplated which enhance levels of stem cells and/or facilitate the angiogenic or vasculogenic capability of stem cells or their precursors or early committed cells of a particular cell lineage or to mobilize stem cells to thereby promote repair and maintenance of elements and components of the systemic vasculature. A method is also provided to activate hypoxic gene programming in target tissues to induce angiogenesis and recruit progenitor cells to that site. Still further, a method to facilitate enhancement of immune function is also provided. Agents and pharmaceutical compositions including functional foods and specialized drug delivery devices useful in the treatment and prophylaxis of events, conditions and disease of the systemic vasculature and to enhance the immune system are contemplated.

BACKGROUND

Bibliographic details of the publications referred to by the author in this specification are collected at the end of the description.

The reference to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that the prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Cardiovascular disease is a leading cause of mortality especially in the Western World. According to statistics, around 13.2 million people in the United States suffer from
coronary artery disease (CAD) and 5.5 million suffer from stroke of which 88% is ischemic. Peripheral arterial disease (PAD) is a common disorder whose prevalence depends on whether it is defined by clinical symptoms or the ankle-to-brachial systolic blood pressure index (Thom et al, Circulation, 113:85-151, 2006; Hirsch et al, Circulation, 113:463-654, 2006). In patients with symptoms of claudication, 1.4% may develop ischemic ulcers over a five year period (Fowkes et al, Int J Epidemiol, 20:384-392, 1991). It is estimated that 5 million people in the United States suffer from congestive heart failure (CHF) [Thom et al, 2006 supra] and a study of randomized controlled trials and epidemiological data in heart failure has identified CAD as the cause of heart failure in approximately two thirds of patients (He et al, Arch Intern Med, 161:996-1002, 2001).

Hence, cardiovascular disease and other conditions of the systemic vasculature represent major life threatening and debilitating diseases. To better understand the present invention, the following discussion is provided on various aspects of the systemic vasculature.

Vasculogenesis is a process that occurs in the embryo whereby blood vessels are formed from endothelial progenitor cells (EPCs). This process also contributes to blood vessel formation in the adult (Asahara et al, Science 275(5302):964-7, 1997). Angiogenesis is a distinct process whereby new capillaries sprout from pre-existing vasculature which involves the proliferation of already differentiated progenitor cells. Arteriogenesis is a process whereby existing collaterals remodel to increase in size secondary to changed flow conditions after occlusion of the parent artery (e.g. those observed on angiography). The importance of these processes is abundantly clear when one considers that, on average, cells need to be located within 100-200μm of a blood vessel to survive (Carmeliet and Jain, Nature 407(6801):249-57, 2000).

The term angiogenesis was first used to describe the growth of blood vessels in the placenta by Hertzig in 1935 and later re-introduced by Folkman (Folkman, Ann Surg 175(3):409-16, 1972). The new vessels sprout from the post-capillary venule; the existing endothelial cells are activated and the parent vessel is dilated by nitrous oxide (NO).
Degradation of the basement membrane and extracellular matrix (ECM) from the parent vessel allows endothelial cells to migrate to form endothelialized tubes 5-8μm in diameter that resemble capillaries. Thereafter, smooth muscle cells or pericytes surround these cells to form larger or smaller vessels, respectively and have a passivating effect on the endothelial cells by causing re-differentiation. These supporting cells provide nascent endothelium, inhibit further endothelial proliferation and stimulate the production of ECM. Thereafter, flow remodels the new vessel as it matures. Endothelial cell integrins, α_5β_3 and α_vβ_3, are induced by angiogenic cytokines (Senger et al, Am J Pathol 149(1):293-305, 1996) and antibodies to these integrins or their binding by Arg-Gly-Asp (RGD) peptides inhibit the angiogenic response (Hammes et al, Nat Med 2(5):529-33, 1996; Brooks et al, Science 264(5158):569-71, 1994; Brooks et al, J Clin Invest 96(4):1815-22, 1995). NO plays a vital role in causing the re-differentiation of the migrating endothelial cells by abrogating proliferation and causing the formation of tube-like structures (Babaei et al, Circ Res 82(9):1007-1015, 1998; Cooke and Losordo, Circulation 105(18):2133-2135, 2002). NO is also required for endothelial cell migration and expression of α_vβ_3 (Murohara et al, Arterioscler Thromb Vasc Biol 19(5):1156-1161, 1999; Ziche et al, P. J Clin Invest 94(5):2036-44, 1994). These aspects correlate with clinical observations; hypercholesteremia and oxidized LDL inhibit angiogenic responses (Van et al, Circulation 96(8):2667-2674, 1997; Chen et al, Arterioscler Thromb Vasc Biol 17(7):1303-1312, 1997; Chen et al, Proc Assoc Am Physicians 109(4):351-61, 1997) and in humans, endothelial dysfunction is a predictor of poor outcomes and shows a graded response to the number of cardiac risk factors present (Davignon and Ganz, Circulation 109(23 suppl_1):III-27-32, 2004; Bonetti et al, Arterioscler Thromb Vasc Biol 23(2):168-175, 2003).

Indeed, in a canine model of repeated cardiac ischemia of the LAD artery designed to induce collateralization, the action of VEGF was abrogated with L-NAME (Matsunaga et al, Circulation 102(25):3098-3103, 2000). Arteriogenesis shares similarities with angiogenesis at the cell signal level except for the inciting factor being shear stress resulting in vascular remodelling. Notably, shear stress signals via many of the same mechanisms implicated in angiogenesis; for example shear induces basic fibroblast growth factor (bFGF) release via a α_5β_3-mediated pathway (Gloe et al, J. Biol. Chem.

A major limitation of the angiogenic therapeutic modalities that have been tried to date is that they have concentrated on a single angiogenic factor instead of a balance of multiple angiogenic factors (Bruick and McKnight, *Genes Dev.* 15(19):2497-2502, 2001). Animal studies, undertaken before human trials, suggested that these therapeutic approaches were not going to succeed. For example, in transgenic mice with VEGF expression driven by the keratin 14 (K14) promoter, the skin showed an increased number of blood vessels (Thurston et al, *Science* 286(5449):2511-4, 1999). These vessels were leaky. However, when VEGF and ANG1 were expressed from the K14 promoter, an augmented angiogenic response was seen and the vessels were not leaky, resistant to leakage by inflammatory agents and had an increased diameter compared to those produced by VEGF over-expression (Thurston *et al*, 1999 *supra*).

Metabolism in muscular tissues is supported by the delivery of nutrients and oxygen by blood vessels. The metabolic rate of muscle tissue and capillary density has been correlated in various species. In a rabbit study, hypoxia has been shown to be necessary for angiogenesis and wound healing and that exposing rabbits to high oxygen concentrations depressed angiogenesis and the wound healing response (Knighton *et al*, *Surgery* 90(2):262-70, 1981). Further, capillary density in rabbit skeletal muscle preparations correlated with electrical stimulation of the muscle and metabolic activity (Hudlicka *et al*, *Am J Physiol* 243(4):H528-35, 1982). The body has, therefore, a hypoxia detection system that has the capacity to detect oxygen concentrations in the microenvironment and to direct the growth of new blood vessels into oxygen-poor areas. Endothelial cells have been shown to respond to hypoxia by up-regulating platelet-derived growth factor B (PDGF-B) transcription (Kourembanas *et al*, *J Clin Invest* 86(2):670-4, 1990) and cultured cell lines have been shown to respond to hypoxia by upregulating VEGF gene expression (Shweiki *et al*, *Nature* 359(6398):843-5, 1992). Furthermore, hypoxia increases Flt-1 (an endothelial VEGF receptor) expression on human umbilical vascular endothelial cells (HUVECs) and
hence hypoxia also appears to regulate the capacity of tissues to respond to VEGF (Gerber et al., J Biol Chem 272(38):23659-67, 1997). Although originally there was controversy regarding the controller of angiogenesis, i.e. a ‘metabolic’ hypothesis was advanced, as was an ‘oxygen-sensing’ hypothesis (Adair et al., Am J Physiol 259(3 Pt 2):R393-404, 1990), it later became clear that oxygen itself controls the response to hypoxia. Clues to determine whether angiogenesis is regulated by ‘metabolic’ or 'hypoxic' stimuli can be inferred by studying the stimuli that cause an increase in erythropoietin (EPO). EPO responds briskly to hypoxia; circulating levels increase hundred fold within hours of hypoxic stimulation (Jelkmann, Physiol Rev 72(2):449-89, 1992). Moreover, early studies showed EPO to be unresponsive to cyanide which uncouples mitochondrial oxidative phosphorylation and thereby interferes with cell metabolism (Necas and Thorling, Am J Physiol222(5):1187-90, 1972). However, erythropoietin responds to cobalt (Goldwasser et al, Blood, 13:55-60, 1958).

It has now been determined that both erythropoiesis and angiogenesis are linked by common molecular switches, at the centre of which is hypoxia inducible factor 1 (HIF-1) (Gleadle et al, Am J Physiol 268(6 Pt 1):C1362-8, 1995; Forsythe et al, Mol Cell Biol 16(9):4604-13, 1996; Goldberg and Schneider, J Biol Chem 269(6):4355-9, 1994).

The human EPO gene has been introduced into mice and four nuclear factors identified which bind to the EPO promoter and enhance gene expression; two of these factors were shown to be dependent on hypoxia (Semenza et al, Proc Natl Acad Sci U S A 88(13):5680-4, 1991). Subsequent work identified a nuclear factor that was induced by hypoxia via de novo protein synthesis as being required for upregulation of EPO expression; this factor was named HIF-1 (Semenza and Wang, Mol Cell Biol 12(12):5447-54, 1992). HIF-1 was subsequently purified and characterized in a human hepatic cell line and in other cultured cell lines where it was shown to be induced by hypoxic conditions (Wang and Semenza, J Biol Chem 270(3):1230-7, 1995; Semenza, Annu Rev Cell Dev Biol 15:551-78, 1999). In human hepatic cells, HIF-1α was detectable 30 mins after cell exposure to 1% v/v oxygen and peaked after 4-8 hours of continuous hypoxia. Upon returning cells to 20% v/v oxygen, HIF-1α decayed within 5 minutes (Wang and Semenza 1995 supra). HIF-1 was
found to be composed of two subunits: a 120 kDa HIF-α subunit and a 91-94kDa HIF-β subunit.

A high oxygen concentration down-regulates HIF activity via an increase in protein destruction and a decrease in co-activator binding. HIF-1β is constitutively expressed at a basal rate in the nucleus and is not responsive to hypoxia (Semenza et al, 1999 supra; Huang et al, Subunit. J. Biol. Chem. 271(50):32253-32259, 1996). In contrast, HIF-1α is induced by hypoxia and interacts with hypoxia response elements (HREs) to induce transcriptional activity (Wiesener et al, Blood 92(7):2260-2268, 1998; Tian et al, Genes Dev 11(1):72-82, 1997).

Knockout mice of HIF-1 offer some insight into the role of HIF-1 in normal physiology. Homozygous HIF-1α knockout results in embryonic lethality at mid-gestation (Ryan et al, Embo J 1998;17(11):3005-15, 1998). This result is not surprising given that HIF-1α-deficient stem cells do not induce VEGF mRNA in response to hypoxia and mutant stem cell tumors do not form large blood vessels (Carmeliet et al, Nature 394(6692):485-90, 1998; Iyer et al, Genes Dev. 12(2):149-162, 1998). Mice heterozygous for HIF-1α (Hif1α+/−) do not develop polycythemia in response to 10% v/v for O₂ as did their wild-type Hif1α+/- littermates. Further, Hif1α+/− mice failed to develop pulmonary hypertension, remodelling of pulmonary arterioles or cor pulmonale as the wild type mice did (Yu et al, J Clin Invest 103(5):691-6, 1999). Further, the pulmonary smooth muscle cells from heterozygous knockout mice were not able to undergo hypoxia-induced hypertrophy as did cells from wild type animals (Shimoda et al, Am J Physiol Lung Cell Mol Physiol 281(1):L202-8, 2001). Also, endothelin-1 is regulated by HIF-1 in lungs (Hu et al, Biochem Biophys Res Commun 245(3):894-9, 1998) and the absence of pulmonary hypertension in knockout mice is consistent with human pulmonary hypertension where endothelin antagonists are efficacious therapy.

In response to hypoxia, HIF proteins bind to the HRE of the VEGF gene and thereby up-regulate gene expression (Forsythe et al, 1996 supra). Also, the VEGF receptor is regulated by HIF-1 as are a number of other genes.
The role of HIF-1 in angiogenesis in myocardium has been illustrated. VEGF mRNA has been shown to be increased after hypoxia in both cultured neonatal myocardial cells and in vivo when pigs had repeated occlusion of the LAD artery (Banai et al, Cardiovasc Res 28(8):1176-9, 1994). Furthermore, VEGF protein is increased 4.5-fold, VEGF mRNA 3.2-fold, and HIF-1α protein 3.8-fold in ventricular tissue from anemic fetuses (Martin et al, Am J Obstet Gynecol 178(3):527-34, 1998). This illustrates how hypoxia can regulate HIF-1 levels, presumably by inhibition of the degradation pathways for HIF-1; HIF-1 can then up-regulate VEGF gene expression and hence increase protein levels. Given the central dual role of HIF-1 in regulating VEGF levels and VEGF response, it is an ideal drug target for controlling clinical angiogenesis.

Since HIF-1 signals downstream to VEGF, it might be expected that HIF-1 signaling is up-regulated prior to a VEGF response under hypoxic conditions. Indeed, HIF-1α mRNA is up-regulated in human cardiac myocytes in response to ischemia in the preceding 24 hours and that this was followed by a sustained (for up to 120 hours) up-regulation of VEGF mRNA (Lee and Wolf, N Engl J Med 342(9):626-633, 2000). Interestingly, in this study, HIF-1α was upregulated in cardiac myocytes and endothelial cells whereas VEGF was upregulated in endothelial cells only.

Given the efficacy of a constitutively active HIF-1α in inducing angiogenesis in transgenic mice, a construct was created from constitutively active HIF-1α and the VP16 transactivation domain from the herpes virus (Vincent et al, Circulation 102(18):2255-2261, 2000). Naked DNA transfection studies confirmed upregulation of endogenous VEGF gene expression in HeLa and C6 cells as well as both VEGF and EPO in Hep3B cells independently of hypoxia. In a rabbit model of hind limb ischemia, intramuscular administration of the HIF-1α/VP16 plasmid was associated with significant improvements in calf blood pressure ratio, angiographic score, resting and maximal regional blood flow, and capillary density (Vincent et al, 2000 supra). This study showed that transfection with HIF-1α/VP16 resulted in gene expression that was similar to that of "natural hypoxia". Using human fetal cardiac myocytes, transfection with constitutively active HIF-1α was
shown to result in a similar pattern of gene expression as that induced by 1% v/v oxygen for 8 hours (Jiang et al, *Physiol. Genomics* 8(1):23-32, 2002). Hence, “turning on” HIF-1α results in physiologically relevant angiogenesis. In addition to having evidence for efficacy in an animal hindlimb ischemia mode, the HIF-1α/VP16 construct also has efficacy in animal models of myocardial infarction (MI). Indeed, when naked DNA was injected into the infarct area after LAD artery ligation, the treated rats had a reduction in infarct size and an increase in capillary density compared to controls (Shyu et al, *Cardiovasc Res* 54(3):576-83, 2002).

Current first line therapies for coronary artery disease involve risk factor control, symptom relief and revascularization where appropriate (Gibbons et al, *J Am Coll Cardiol* 41:159–168, 2003). Medical therapies used include lipid lowering therapies (e.g. statins), beta-blockers, antiplatelet agents (aspirin, clopidogrel) and angiotensin converting enzyme inhibitors. In addition, risk factors such as obesity, diabetes, hypertension and smoking are managed appropriately. In patients suffering from acute coronary syndromes, many of these same treatments are also employed but with the addition of dual antiplatelet therapy with (aspirin and clopidogrel) as well heparin or other antithrombotics combined with early percutaneous coronary intervention (PCI) (Braunwald et al, *J Am Coll Cardiol* 40:1366-1374,2002; Antman et al, *J Am Coll Cardiol*, 44:671-719, 2004). Further, thrombolytic agents are recommended for ST-elevation when timely PCI is not available. These established therapies do not target HIF proteins.

Current first line therapies for ischemic stroke include thrombolytic agents, but these do not target HIF proteins. The thrombolytic therapies have a high risk of causing intracranial hemorrhage and must be administered within three hours of the onset of stroke to be effective. Second line defences include anti-coagulants where appropriate and anti-platelet agents which stop the formation of blood clots and inhibit their growth.

CHF is a disorder with a lifetime prevalence of approximately 20% and a 5 year mortality rate that approaches 50%. As previously mentioned, ischemic heart disease accounts for about two thirds of heart failure and the treatments for this condition include beta blockers,

These conditions described above share a common core pathophysiologic process which can be described as "atherothrombosis" which is often diffuse and affects more than one vascular bed (Juan *et al*, *Eur Heart J.*, 25(14):1197-1207, 2004). In addition to some of the medical therapies mentioned above, surgical and percutaneous revascularization plays a role in the management of these ischemic diseases. About 1.4 million operations arterial bypass operations are performed annually in the United States alone. Furthermore, in about one hundred thousand patients, this treatment is not an option as no suitable autologous artery or vein exists (McKee *et al*, *EMBO*, 4:633-638, 2003; Lloyd-Jones *et al*, *Circulation*, 106:3068-3072, 2002; American Heart Association, *Heart and Stroke Statistical Update*, 2002). Indeed, in 2003, 467,000 coronary artery bypass procedures were performed and 1,244,000 coronary angioplasties were performed (Heart Disease and Stroke Statistics Update, 2006). These therapies are not perfect; PCI provides symptomatic relief but does not improve long term patient outcomes in non-acute coronary disease (Katritsis *et al*, *Circulation*, 111: 2906-12, 2005).

Given the limitations with current therapies, there has been interest in developing "angiogenic therapies" to treat ischemic diseases. Current, gene therapy based angiogenic therapies allow sustained production of angiogenic factors resulting in prolonged (but limited) exposure to elevated levels from a single, locally delivered administration. Gene therapy also allows angiogenic factor production and secretion to be directed to a specific cell type. Nevertheless, the safety of such viruses that incite an inflammatory response is uncertain (Simmons *et al*, *Circulation*, 102:E73-86, 2000; Muhlhauser *et al*, *Gene Therapy*, 3: 145-153, 1996). Current therapies also possess numerous other disadvantages such as requiring the introduction of foreign genetic material. The patient is also exposed to viral vectors with the concomitant risk of inflammatory response, viral persistence and *in vivo* recombination. Such methods also raise the potential for non-target cell gene delivery and current vector delivery systems do not allow precise modulation of angiogenic factor expression levels. It is also possible that the therapy may be inactivated by inflammatory
response, especially when readministration is required. Finally, the patient may be at risk of being systemically exposed to secreted angiogenic factors in the long term (Simmons et al, Circulation, 102:E73-86, 2000). Gene therapy trials using either adenoviral vectors or the direct injection of naked plasmids into the myocardium employing FGF-4 or VEGF<sub>165</sub> coding sequences have been undertaken. The data resulting from such trials do not show clear evidence of efficacy of such treatments (Grines et al, Circulation, 105: 1291-1297, 2002; Hedman et al, Circulation, 107: 2677-2683, 2003; Losordo et al, Circulation, 105: 2012-2018, 2002). Studies have also demonstrated that gene therapy based approaches exhibit problems associated with drug delivery. In particular, animal studies have shown low rates of myocardioocyte infection after adenoviral gene therapy via the intracoronary infusion (Mühlhauser et al, Gene Therapy, 3: 145-153, 1996). Methods available for enhancing transfection rates are not acceptable in a clinical setting (Wright et al, Gene Therapy, 8:1833-1839, 2001). Even if an ideal protein therapy or vector for gene therapy could be produced, delivery would still be an issue since even direct myocardial injection does not result in complete retention of injectate (Grossman et al, Catheter Cardiovasc Interv, 55: 392-397, 2002). Further, when protein or gene based therapies in concentrations sufficient for therapeutic angiogenesis are used, side effects occur secondary to systemic exposure, namely hypertension and edema with VEGF and anemia, thrombocytopenia and renal toxicity with FGF (Freedman et al, Ann Intern Med, 136: 54-71, 2002).

Current protein therapies allow titratable dosing of angiogenic factors resulting in finite temporal exposure. Nevertheless, protein therapies have a short serum half life, finite tissue half life and only allow very short periods of exposure. As a result repeated administration is often required (Simmons et al, 2000 supra). Prolonged infusions of intra-atrial or intracoronary VEGF or FGF which lasted for longer than 28 days were required to have an effect on collateral developments and flow in dog models of left anterior descending artery occlusion (Banai et al, Circulation, 89: 2183-2189, 1994; Lazarous et al, Circulation, 91: 145-153, 1995). Indeed, single boluses were ineffective in the dog models of myocardial infarction and prolonged infusions would not be practical in a clinical setting. In humans, clinical trials with either protein based therapy or gene based therapy have generally shown little effect (Freedman et al, Ann Intern Med, 136: 54-71, 2002). Clinical trials with
intravenous or intracoronary infusions of VEGF and FGF-2 protein have not yielded favourable results in CAD patients except for improvement in symptom class reported in some studies (Henry et al., Circulation, 107:1359-1365, 2003; Simmons et al., Circulation, 105: 788-793, 2002). Likewise, symptomatic improvement with intra-arterial FGF2 infusions in patients suffering from claudication due to peripheral vascular disease did not persist at a 6 month follow up (Lederman et al., Lancet, 359:2053-2058, 2002). Studies that simply infuse peptide into a blood vessel are associated with particularly poor retention of protein in the heart. In pigs 0.88% of radio labelled FGF given by intracoronary infusion was retained at 1 hour and this had dropped to 0.05 at 24 hours. Myocardial retention is even lower when intravenous infusion is used (Laham et al., Drug Metab Dispos, 27: 821-826, 1999).

Cell based therapies and cell-mobilizing therapies have also mixed results without a clear indication of efficacy (Welt et al., Circulation, 113:1272-1274, 2006). These trials have included intracoronary infusion of autologous bone marrow aspirate or the use of granulocyte-colony stimulating factor (G-CSF) or granulocyte-monocyte colony stimulating factor (GM-CSF) to mobilize stem cells from the bone marrow post-infarct. Indeed, progenitor cells are mobilized in patients suffering from an acute myocardial infarction (Wojakowski et al., Circulation, 110: 3213-3220, 2004; Massa et al., Blood, 105: 199-206, 2005; Shintani et al., Circulation, 103: 2776-2779, 2001) and stroke (Ghani et al, Stroke, 36:151-153, 2005); the level of these stem cells has been shown to be a predictor of global and regional improvement in left-ventricular function (Leone et al, Eur Heart J, 26: 1196-1204, 2005). Strategies employed to augment this natural response have included the use of G-CSF to mobilize bone marrow cells and the isolation of progenitor cells, either from peripheral blood or bone marrow aspirate for intracoronary infusion or intramyocardial injection. Despite proof of beneficial effects on post-infarct remodelling in studies that used intracoronary infusion of progenitor cells (Assmus et al, Circulation, 106: 3009-3017, 2002; Wollert et al, Lancet, 364: 141-148, 2004; Schachinger et al, J Am Coll Cardiol, 44: 1690-1699, 2004), this benefit did not persist on long term follow up in one of the studies (Meyer et al, Circulation, 113: 1287-1294, 2006). In a small 20 patient trial with direct intramyocardial delivery of bone marrow mononuclear cells, improvements

Hence, gene therapy is not a practical way of targeting HIF-1.

Accordingly, there is a need to develop effective treatments for cardiovascular and ischemic disease and other events and conditions of the systemic vasculature which overcome problems associated with current treatments relating *inter alia* to the restricted treatment window, risk of bleeding and lack of suitability in some patients. In particular, there is a need to develop strategies for promoting HIF-1 levels or stability in order to promote angiogenesis and other physiological processes involved in the systemic vasculature.
SUMMARY OF THE DISCLOSURE

A method is provided for the treatment and prophylaxis of events, conditions and diseases of the systemic vasculature by increasing stem cells numbers and recruiting them to sites of damage and facilitating repair and maintenance of elements of the systemic vasculature. This has the effect of decreasing overall cardiovascular risk prior to or following the event, condition or disease of the systemic vasculature. Enhancement of immune function is also contemplated and hence one aspect of the method is useful in the treatment and prophylaxis of infection. The methods described herein are predicated in part on the determination that particular agents such as certain transition metals, compounds containing these transition metals, hydralazine and hydralazine-related compounds increase stem cell numbers, enhance the angiogenic capacity of stem cells, activate hypoxic gene programming in target tissues and enhance angiogenic responses and stem cell mobilization to particular sites. More particularly, the agents contemplated herein stabilize or otherwise enhance levels of HIF-1. The latter also facilitates and stimulates immune function, hence, elevating levels of HIF-1 is useful in treating or preventing infection by pathogenic agents.

The generation of new tissue or the regeneration of damaged tissue of the systemic vasculature by adult or embryonic stem cells provides therapeutic advantages in treating cardiovascular disease and ischemia. Stem cell-aided regeneration and repair of ischemic or necrotic tissue enables enhanced functional re- or neo-vascularization.

Hence, methods, agents, compositions and uses are provided. Accordingly, in one aspect, a method is contemplated for the treatment or prophylaxis of an event, condition and/or disease of, or affecting, the systemic vasculature in a subject, said method comprising administering to said subject an effective amount of an agent or combination of agents which elevate levels of stem cells or precursor cells thereof or early committed cells of a particular cell lineage and which enhance angiogenic capability of said cells. In oen form, the agent mobilizes the cells to a site in the systemic vasculature.
Such a method may also be conducted in combination with other treatments including the use of thrombolytic agents, anti-coagulants, anti-platelet agents and/or surgery such as bypass surgery.

In an embodiment, the stem cells are EPCs or precursor cells thereof, or early committed cells in an EPC lineage.

Hence, in another aspect, a method is provided for the treatment or prophylaxis of an event, condition and/or disease of, or affecting, the systemic vasculature in a subject, said method comprising administering to said subject an effective amount of an agent or combination of agents which elevate levels of EPC or precursor cells thereof or early committed cells of a particular lineage and which enhance the angiogenic capability of said cells, and optionally which also mobilize said cells to a site in the systemic vasculature.

Agents contemplated herein include transition metals and compounds containing transition metals such as but not limited to cobalt or pharmaceutically acceptable salts thereof. In addition, hydralazine or hydralazine-related compounds may be used. In essence, the compound is selected on the basis of having the same or similar redox potential as, and can displace, iron in hemoglobin and/or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase.

Hence, in a further aspect, a method is disclosed for the treatment or prophylaxis of an event, condition and/or disease of, or affecting, the systemic vasculature in a subject, said method comprising administering the said subject an effective amount of an agent selected from this list consisting of a compound or transition metal which has the same or similar redox potential as, and can displace, iron in hemoglobin or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal, hydralazine and a hydralazine-related compound said agent elevating levels of EPCs or
cells of EPC lineage and enhancing the angiogenic capability of said cells.

As indicated above, one transition metal is cobalt, which, along with other agents contemplated herein mobilizes cells to a site in the systemic vasculature.

5 Conditions encompassed herein include but are not limited to CAD, cardiac hypertrophy, ischemic stroke, anemia, ulcer or wound and PAD in any subject such as but not limited to human subjects. In one aspect, the instant method is useful for promoting angiogenesis.

10 Accordingly, a method is provided for promoting angiogenesis in a subject said method comprising administering to said subject an effective amount of an agent or combination of agents which elevate levels of stem cells or precursor cells thereof or early committed cells of a particular lineage and which enhance angiogenic capability of said cells and which optionally mobilize said cells to a site in the systemic vasculature.

15 In another aspect, a method is contemplated for the treatment or prophylaxis of infection by a pathogenic agent said method comprising administering to said subject an effective amount of an agent or combination of agents which elevate levels of stabilizing HIF-1.

20 Still a further aspect is directed to the use of an agent which elevates levels of stem cells, precursor cells thereof or early committed cells of a particular cell lineage and which enhances the angiogenic capability of said cells and/or which optionally mobilize said cells to a site within the systemic vasculature in the manufacture of a medicament for the treatment of an event, condition and/or disease of the systemic vasculature or the treatment or prevention of infection.

As indicated above, the agent is *inter alia* a transition metal such as cobalt, a compound comprising same, hydralazine or a hydralazine-related compound.

25 Hence, the use of cobalt, a compound comprising cobalt, hydralazine or a hydralazine-related compound is contemplated in the manufacture of a medicament for the treatment of
an event, condition or disease of the systemic vasculature such as but not limited to CAD, cardiac hypertrophy, ischemic stroke, anemia, ulcer or wound and/or PAD.

Another aspect is the use of cobalt, a compound comprising cobalt, hydralazine or a hydralazine-related compound in the manufacture of a medicament for the treatment of an infection.

A catheter or stent or other mechanical or specialized delivery system is described comprising a releasable agent which elevates levels of stem cells or early committed cells of a particular lineage and which enhances the angiogenic capability of said cells and/or which mobilizes said cells to a site within the systemic vasculature.

Other slow release formulations are also contemplated herein.

Still a further aspect is directed to functional foods comprising transition metals and/or hydralazine in an amount useful for promoting elevated levels of stem cells, such as EPCs. The agents may be naturally part of the food or may be included as an additive. Hence, dietary intervention in the treatment of events, conditions and/or diseases of the systemic vasculature is also provided herein.

A list of abbreviations used in the present specification is provided in Table 1.
**TABLE 1**

*Abbreviations*

<table>
<thead>
<tr>
<th>ABBREVIATION</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>bFGF</td>
<td>Basic fibroblast growth factor</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>CHF</td>
<td>Congestive heart failure</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>EPC</td>
<td>Endothelial progenitor cells</td>
</tr>
<tr>
<td>EPO</td>
<td>Erythropoietin</td>
</tr>
<tr>
<td>FGF</td>
<td>Fibroblast Growth Factor</td>
</tr>
<tr>
<td>HIF-1</td>
<td>Hypoxia inducible factor 1</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>α-subunit of hypoxia inducible factor 1</td>
</tr>
<tr>
<td>HIF-1β</td>
<td>β-subunit of hypoxia inducible factor 1</td>
</tr>
<tr>
<td>HRE</td>
<td>Hypoxia response elements</td>
</tr>
<tr>
<td>HUVECs</td>
<td>Human umbilical vascular endothelial cells</td>
</tr>
<tr>
<td>LAD artery</td>
<td>Left anterior descending artery</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoproteins</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>PAD</td>
<td>Peripheral disease arterial disease</td>
</tr>
<tr>
<td>PCI</td>
<td>Percutaneous Coronary Intervention</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet-derived growth factor</td>
</tr>
<tr>
<td>PDGF-B</td>
<td>Platelet-derived growth factor-B</td>
</tr>
<tr>
<td>PHD</td>
<td>Prolyl-hydroxyl domain</td>
</tr>
<tr>
<td>SSRE</td>
<td>Shear stress response element</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
</tbody>
</table>
DETAILED DESCRIPTION

The following definitions and clarifications are provided.

Unless otherwise indicted, the subject invention is not limited to specific formulation components, manufacturing methods, dosage regimens, or the like, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

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 element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

It must be noted that, as used in the subject specification, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to "a therapy" includes a single therapy or two or more therapies; reference to "an agent" includes a single agent, as well as two or more agents; reference to "the invention" includes a single or multiple aspect of an invention; and so forth.

All scientific citations, patents, patent applications and manufacturer's technical specifications referred to hereinafter are incorporated herein by reference in their entirety.

The terms "agent", "compound", "chemical agent", "pharmacologically active agent", "medicament", "active" and "drug" are used interchangeably herein to refer to a chemical compound that induces a desired pharmacological and/or physiological effect. The terms also encompass pharmaceutically acceptable and pharmacologically active ingredients of those active agents specifically mentioned herein including but not limited to salts, esters, amides, prodrugs, active metabolites, analogs and the like. When the terms "agent", "compound", "chemical agent", "pharmacologically active agent", "medicament", "active", and "drug" are used, then it is to be understood that this includes the active agent per se as
well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, prodrugs, metabolites, analogs, etc.

Reference to an "agent", "compound", "chemical agent", "pharmacologically active agent", "medicament", "active" and "drug" includes combinations of two or more agents. A "combination" also includes multi-part such as a two part composition where the agents are provided separately and given or dispensed separately or admixed together prior to dispensation. For example, a multi-part pharmaceutical pack may have two or more compounds separately maintained. A "combination" also includes an agent impregnated in a matrix, polymer or other solid or semisolid carrier including a catheter, stent or other special release device.

In addition, combination therapy is contemplated herein. Hence, the method described herein may be practiced in combination with existing methods such as thrombolytic treatments, treatment with anti-coagulants or anti-platelet agents, bypass surgery or the introduction of catheters or stents as well as gene therapy. In addition, the agents may be impregnated within or on matrices such as bandages or wound patches.

The desired pharmacological or physiological properties include elevating levels of stem cells, increasing the angiogenic capability of the stem cells and/or mobilizing the stem cells to a site within the systemic vasculature. This has the effect of facilitating repair or maintenance of tissue of the systemic vasculature. Generally, but in no way intending to limit the present invention to any one theory or mode of action, the desired agents of the present invention are proposed to stabilize HIF-1 or otherwise increase HIF-1-mediated gene expression which facilitates an increase in SDF-1 gene expression and other angiogenesis-associated genes including but not limited to EG-VEGF, ENG, LEP, LRP1, TGF-β3 and VEGF. Elevated HIF-1 also facilitates enhancement of the immune system (see for example Cramer et al, Cell 112:645-657, 2003) and hence, the method described herein is useful in the treatment or prophylaxis of infection by a pathogenic agent.

The transition metal useful in the practiced of the present invention is a metal which has
the same redox potential as, and can displace, iron in hemoglobin and/or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron atom dependent 2-oxoglutarate dependent dioxygenase enzyme. This leads to an increase in stem cell numbers and recruits stem cells to the site of damage or potential damage facilitating repair and maintenance. The "iron atom dependent 2-oxoglutarate dependent dioxygenase enzyme" includes any such enzyme including, but not limited to PHD and/or FIH.

A "transition metal" is an element in the d-block of the periodic table, including zinc, cadmium and mercury, corresponding to groups 3 to 12 on the periodic table and/or any element with an incomplete d subshell or that may form stable ions only with an incomplete d subshell. Transitional metals include, but are not limited to, cobalt and a cobalt containing compound, any manganese containing compound, and nickel containing compound.

Reference to "cobalt" includes cobalt or cobalt containing compound such as cobalt (II) acetate, cobalt (II) acetate tetrahydrate, cobalt (II) acetylacetonate, cobalt (ii) acetylacetonate hydrate, cobalt (III) acetylacetonate, cobalt (III) benzoylacetonate, cobalt boride, cobalt (II) bromide, cobalt (II) bromide hydrate, cobalt (II) carbonate hydrate, cobalt (II) chloride, cobalt (II) chloride hydrate, cobalt (II) chloride hexahydrate, cobalt (II) 2-ethylhexanoate, cobalt (II) fluoride, cobalt (II) fluoride tetrahydrate, cobalt (III) fluoride, cobalt (II) iodide, cobalt (II) 2,3-naphthalocyanine, cobalt (II) nitrate hexahydrate, cobalt (II) oxalate dihydrate, cobalt (II) oxide, cobalt (II,III) oxide, cobalt (II) perchlorate hexahydrate, cobalt (II) phthalocyanine, cobalt (II) selenide, cobalt (III) sephulcrate trichloride, cobalt (III) sephulcrate tris (tetraphenylborate), cobalt (II) sulfate hydrate, cobalt (II) tetrafluoroborate hexahydrate, cobalt (II) tetrathiocyanatomercuroate, cobalt (II) thiocyanate, cobalt (II) thiocyanate hydrate, cobalt tris (2,2,6,6-tetramethyl-3,5-heptanedioate), cobalt (II) tungstate hydrate and hydroxycobalamin (Vitamin B12).
Reference to "manganese" includes manganese, manganese(II) acetate, manganese(II) acetate tetrahydrate, manganese(III) acetate dihydrate, manganese(II) acetyladactonate, manganese(III) acetyladactonate, manganese(II) bromide, manganese(II) bromide tetrahydrate, manganese carbonyl, manganese(II) chloride, manganese(II) chloride tetrahydrate, manganese(II) cyclohexanbutyrate, manganese dioxide, manganese(II) fluoride, manganese(III) fluoride, manganese(II) hexafluoroacetyladactonate trihydrate, manganese(II) hydrogen phosphite, manganese(II) hypophosphite monohydrate, manganese(II) iodide, manganese(II) nitrate hydrate, manganese(II) nitrate, manganese(II) oxide, manganese(II, III) oxide, manganese (III) oxide, manganese(IV) oxide, manganese(IV) oxide, 50% w/w on activated carbon, manganese(II) perchlorate hexahydrate, manganese(II) phthalocyanine, manganese(III) phthalocyanine chloride, manganese sesquioxide, manganese(II) sulfate hydrate, manganese(II) sulfate monohydrate, manganese(II) sulfide, manganese(III) 5,10,15,20-tetra(4-pyridyl)-21 H ,23 H –porphine chloride tetrakis(methochloride), manganic acetyladactonate, manganocene and manganous acetyladactonate.

Reference to "nickel" includes nickel, nickel(II) acetate tetrahydrate, nickel(II) acetyladactonate, nickel(II) bis(2,2,6,6-tetramethyl-3,5-heptanedionate), nickel boride, nickel(II) bromide, nickel(II) bromide hydrate, nickel(II) bromide ethylene glycol dimethyl ether complex, nickel(II) bromide 2-methoxyethyl ether complex, nickel(II) carbonate, basic, nickel(II) carbonate hydroxide tetrahydrate, nickel(II) chloride, nickel(II) chloride hexahydrate, nickel(II) chloride hydrate, nickel(II) cyclohexanbutyrate, nickel(II) 2-ethylhexanoate, nickel(II) fluoride, nickel(II) fluoride tetrahydrate, nickel(II) formate dihydrate, nickel(II) hexafluoroacetyladactonate hydrate, nickel(II) hydroxide, nickel(II) iodide, nickel(II) nitrate hexahydrate, nickel(II) 5,9,14,18,23,27,32,36-octabutoxy-2,3-naphthalocyanine, nickel(II) 1,4,8,11,15,18,22,25-octabutoxy-29H,31 H –phthalocyanine, nickel(II) octanoate hydrate, nickel, ~60% w/w on kieselghur, nickel, ~65% w/w on silica/alumina, nickel(II) oxalate dihydrate, nickel(II) oxide, nickel(II) perchlorate hexahydrate, nickel peroxide hydrate, nickel phosphide, nickel(II) phthalocyanine, nickel(II) phthalocyaninetetraculfonylic acid, tetrascumium salt, nickel(II) sulfamet tetrahydrate, nickel(II) sulfate heptahydrate, nickel(II) sulfate hexahydrate, nickel sulfide,

A hydralazine-related compound includes structurally similar compounds which complex to an inorganic subunit of an iron-dependent dioxygenase.

A stem cell includes embryonic stem cells, somatic stem cells, germ stem cells, mammalian epidermal stem cells, adipose derived stem cells, adult neural stem cells, mammalian neurons, mammalian astrocytes, mammalian keratinocyte stem cells, mammalian keratinocyte transient amplifying cells, mammalian melanocyte stem cells, mammalian melanocytes, mammalian foreskin fibroblasts, mammalian duct cells, mammalian pancreatic islets, mammalian pancreatic β-cells, mammalian adult renal stem cells, mammalian embryonic renal epithelial stem cells, mammalian kidney epithelial cells, mammalian hepatic oval cells, mammalian hepatocytes, mammalian bile duct epithelial cells, mammalian embryonic endodermal stem cells, mammalian adult hepatocyte stem cells (existence controversial), mammalian mammary epithelial stem cells, bone marrow-derived stem cells, mammalian lung fibroblasts, mammalian bronchial epithelial cells, mammalian alveolar type II pneumocytes, mammalian skeletal muscle stem cells (satellite cells), mammalian cardiomyocytes, bone marrow mesenchymal stem cells, simple squamous epithelial cells, descending aortic endothelial cells, aortic arch endothelial cells, aortic smooth muscle cells, limbal stem cells, corneal epithelial cells, CD34+ hematopoietic stem cells, mesenchymal stem cells, osteoblasts (precursor is mesenchymal stem cell), peripheral blood mononuclear progenitor cells (hematopoietic stem cells), osteoclasts (precursor is above cell type), stromal cells, mammalian splenic precursor stem cells, mammalian splenocytes, mammalian CD4+ T-cells, mammalian CD8+ T-cells, mammalian NK cells, mammalian monocytes, mammalian macrophages, mammalian dendritic cells, mammalian B-cells, goblet cells (mucus secreting cells of the nose), pseudostratified ciliated columnar cells (located below olfactory region in the nose), pseudostratified ciliated epithelium (cells that line the nasopharyngeal tubes), stratified epithelial cells (cells that line and structure the trachea), ciliated columnar cells (cells that
line and structure the trachea), goblet cells (cells that line and structure the trachea), basal cells (cells that line and structure the trachea), cricopharyngeus muscle cells, female primary follicles or male spermatogonium, endothelial progenitor cells, vascular progenitor cells, hemangioblast cells and fetal stem cells derived from umbilical cord blood. The preferred mammalian cells are human cells.

Particular stem cells include endothelial progenitor cells or "EPCs" or any early committed cell in the EPC lineage or precursor of an EPC, and in particular human EPCs.

The terms "effective amount" and "therapeutically effective amount" of an agent as used herein mean a sufficient amount of the agent (e.g. a transition metal or transition metal containing compound) to provide the desired therapeutic or physiological effect or outcome. Undesirable effects, e.g. side effects, are sometimes manifested along with the desired therapeutic effect; hence, a practitioner balances the potential benefits against the potential risks in determining what an appropriate "effective amount" is. The exact amount required will vary from subject to subject, depending of the species, age and general condition of the subject, mode of administration and the like. Thus, it may not be possible to specify an exact "effective amount". However, an appropriate "effective amount" in any individual case may be determined by one of ordinary skill in the art using only routine experimentation.

The desired therapeutic or physiological effects, as indicated above, is to enhance levels of EPCs (or other stem cells), increase their angiogenic capability and/or to mobilize the stem cells to a particular site in the systemic vasculature. In addition, the agent will directly or indirectly regulate levels and/or activity of HIF-1 which in turn has an affect on SDF-1 gene expression or other gene as listed above. Hence, it is proposed herein that the subject agent inactivates prolylhydroxylase domains (PHDs) [which target HIF-1α for destruction] and factors inhibiting HIF proteins (FIHs) [which inhibit HIF-α activity]. Elevated or stabilized levels of HIF such as HIF-1, facilitates enhancement of immune function. Hence, this in turn assists in the treatment or prophylaxis of infection by a pathogenic agent. Examples of pathogenic agents include viruses (e.g. HIV, hepatitis virus, herpes
virus, etc), prokaryotes (enterobacteria, *Pseudomous*, etc) and eukaryotes (e.g. fungi, yeast, etc).

By "pharmaceutically acceptable" carrier, excipient or diluent is meant a pharmaceutical vehicle comprised of a material that is not biologically or otherwise undesirable, i.e. the material may be administered to a subject along with the selected active agent without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, detergents, coloring agents, wetting or emulsifying agents, pH buffering agents, preservatives and the like.

Similarly, a "pharmacologically acceptable" salt, ester, amide, prodrug or derivative of a compound as provided herein is a salt, ester, amide, prodrug or derivative that is not biologically or otherwise undesirable.

The terms "treating" and "treatment" as used herein refer to reduction in severity and/or frequency of symptoms of the condition being treated, elimination of symptoms and/or underlying cause, prevention of the occurrence of symptoms of the condition and/or their underlying cause and improvement or remediation or amelioration of damage following a condition.

"Treating" a subject may involve prevention of a condition or other adverse physiological event in a susceptible individual as well as treatment of clinically symptomatic individual by ameliorating the symptoms of the condition.

A "subject" as used herein refers to an animal, particularly a mammal and more particularly a human who can benefit from the pharmaceutical formulations and methods herein described. There is no limitation on the type of animal that could benefit from the presently described pharmaceutical formulations and methods. A subject regardless of whether a human or non-human may be referred to as an individual, patient, animal, host or recipient. The compounds and methods described herein have application in human
medicine, veterinary medicine as well as in general, domestic or wild animal husbandry. The compositions also have industrial applications.

As indicated above, particular animals are humans, non-human primates such as marmosets, baboons, orang-utans, lower primates such a tupia, livestock animals, laboratory test animals, companion animals or captive wild animals. A human is the most preferred target. However, non-human animal models may be used.

Examples of laboratory test animals include mice, rats, rabbits, guinea pigs and hamsters. Rabbits and rodent animals, such as rats and mice, provide a convenient test system or animal model as do primates and lower primates. Livestock animals include sheep, cows, pigs, goats, horses and donkeys. Non-mammalian animals such as avian species are also contemplated. Instead of a live animal model, a test system may also comprise an in vitro culture system.

Reference to an event, condition or disease of the systemic vasculature includes but is not limited to anemia, coronary artery disease (CAD), cardiac hypertrophy, peripheral arterial disease (PAD), ischemic stroke, peripheral vascular disease, ischemic ulcers or wounds, damage or diseases of the heart, great vessel or coronary vasculature, damage to blood vasculature, atherosclerosis, peripheral vascular disease, coronary heart disease or heart failure, hypertension, isolated systolic hypertension, end stage renal disease, diabetic nephropathy, arteriosclerosis, stiff vessel disease, peripheral vascular disease, coronary stroke, peripheral vascular disease, periarticular rigidity, sickle cell anemia, retinopathy, ischemic heart disease, blockages of the coronary artery system, treatment following a wider range of open surgical and interventional cardiology procedures, occlusion of the vasculature, a hemorrhagic event in the vasculature, thromboembolism, cerebral ischemia, spasms of the coronary vasculature, as a secondary treatment to a cardiopulmonary bypass procedure or to a surgical intervention which is imposed on a subject, vasospasm, transient ischemic attack (TIA), thrombotic occlusion, ischemia, thromboembolic stroke, hemorrhagic stroke, head trauma, cardiac arrest, severe blood loss due to injury or internal hemorrhage and other similar conditions that disrupt normal blood flow, spontaneous
intracerebral hemorrhage (e.g. hypertensive, amyloid angiopathy); ruptured aneurysm (e.g. saccular, mycotic); ruptured arteriovenous malformation, thrombosis; vasculitis (including collagen vascular disease (e.g. temporal (giant cell) arteritis, polyarteritis nodosa, Wegener's granulatosis, Takayasu's arteritis, syphilis), meningitis (e.g. tuberculosis, fungi, syphilis, bacteria, herpes zoster), arterial dissection (e.g. carotid, vertebral, intracranial arteries at the base of the brain), hematologic disorders (e.g. polycythemia, thrombocytosis, thrombotic, thrombocytopenic purpura, disseminated intravascular coagulation, dysproteinemias, hemoglobinopathies (sickle cell disease)) and that caused by cocaine, amphetamines, moyamoya disease, fibromuscular dysplasia and Binswanger's disease); embolism (including cardiac sources (e.g. dysrhythmia, coronary heart disease, rheumatic heart disease, etc.), coronary artery bypass graft (CABG), atherothrombotic arterial sources (e.g. bifurcation of common carotid artery, carotid siphon, distal vertebral artery, aortic arch) and unknown sources (e.g. may be associated with a hypercoagulable state secondary to systemic disease, carcinoma (especially pancreatic), eclampsia, oral contraceptives, lupus, factor C or S deficiency, Factor V mutation such as Factor V Leiden, etc.)); vasoconstriction (including vasospasm (e.g. cerebral vasospasm following subarachnoid hemorrhage) and reversible cerebral vasoconstriction (e.g. idiopathic, migraine, eclampsia, trauma); ischemic renal disease (e.g. secondary to renal artery stenosis) and venous conditions (including dehydration, pncranial infection, postpartum and postoperative states and systemic cancer).

Particularly provided is a treatment and prophylaxis of CAD, cardiac hypertrophy, ischemic stroke and ischemic ulcers and wounds, PAD, anemia and peripheral vascular disease. Cardiac hypertrophy, for example, is a thickening of heart muscle (myocardium) which results in a decrease in the size of the heart chamber, including left and right ventricles. A common cause of cardiac hypertrophy is high blood pressure (hypertension) and heart valve stenosis. However, the present invention extends to cardiac hypertrophy by any cause (see Minamino et al, *Nature* 446:444-448, 2007).

The subject invention is predicated in part on the vessel-forming capacity of stem cells as illustrated by embryonal vasculogenesis in which undifferentiated cells form primitive
blood vessels. In adults, circulating stem cells are incorporated into the vessel wall. The functional potential of EPCs becomes apparent from their ability to contribute to natural mechanisms of blood vessel formation and repair. In addition to the function of EPCs being important, their absolute numbers are also a factor. Indeed, EPCs contribute to ongoing endothelial repair and restore endothelial function. In humans, the number of EPC colony forming units correlates positively with flow mediated branchial artery reactivity and inversely with the Framingham risk score (Hill et al, *N Engl J Med*, 348:593-600, 2003). Furthermore, in patients deemed high risk by the Framingham risk score, a higher rate of the senescent phenotype is observed. The finding of endothelial dysfunction with reduced EPC colony forming units is significant since this surrogate marker is a predictor of poor outcomes and shows a graded response to the number of cardiac risk factor present (Davignon et al, 2004 *supra*; Bonetti et al, 2003 *supra*). Clinical end point study levels of circulating EPCs in patients with angiographic CAD predicted event-free survival (Werner et al., *N Engl J Med*, 353:999-1007, 2005).

Mechanisms of blood vessel formation and repair include angiogenesis, vasculogenesis and arteriogenesis (Zwaginga et al, *Clinical and Experimental Pharmacology and Physiology*, 30:900-908, 2003). Vasculogenesis is a process that occurs in the embryo whereby blood vessels are formed from EPCs. This process also contributes to blood vessel formation in the adult. Angiogenesis is a distinct process whereby new capillaries sprout from pre-existing vasculature and involves the proliferation of already differentiated progenitor cells. Arteriogenesis is a process whereby existing collaterals remodel to increase in size secondary to changed flow conditions after occlusion of the parent artery (Ashara et al, 1997 *supra*; Carmeliet and Jain, 2000 *supra*).

Although not intending to limit the present invention to any one theory or mode of action, it is proposed that the subject agents (e.g. transition metals) modulate the hypoxia detection system and hence simulate ischemia. This assists in the mobilization of stem cells, such as EPCs to the site of action of the agents. This is of therapeutic potential since bone-marrow derived endothelial progenitor cells contribute to post-natal vasculogenesis related to processes such as wound healing and pathologic ischemia to end organs (Asahara T,

In addition, the agents stabilize or enhance levels of HIF-1 which also has a downstream effect on expression of genes involved in angiogenesis. Hence, the angiogenic and vasculogenic potential of stem cells is enhanced.

Accordingly, a method is provided for the treatment or prophylaxis of an event, condition and/or disease of, or affecting, the systemic vasculature in a subject, said method comprising administering to said subject an effective amount of an agent or combination of agents which elevate levels of stem cells or precursor cells thereof or early committed cells of a particular lineage and which enhance angiogenic capability of said cells.

As indicated above, the preferred stem cells are EPCs. Preferably, the agent also mobilizes the cells to a site in the systemic vasculature.

Hence, another aspect contemplates a method for the treatment or prophylaxis of an event, condition and/or disease of, or affecting, the systemic vasculature in a subject, said method comprising administering to said subject an effective amount of an agent or combination of agents which elevate levels of EPC or precursor cells thereof or early committed cells of a particular lineage and which enhance angiogenic and vasculogenic capability of said cells and/or which optionally mobilize said cells to a site in the systemic vasculature.

Particular compounds are selected from the list consisting of a transition metal or compound which has the same or similar redox potential as, and can displace, iron in hemoglobin and/or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a
compound or complex containing said transition metal or compound; hydralazine; and a
hydralazine-related compound.

Accordingly, another aspect provides a method for the treatment or prophylaxis of an
event, condition and/or disease of, or affecting, the systemic vasculature in a subject, said
method comprising administering the said subject an effective amount of an agent selected
from this list consisting of a transition metal or compound which has the same or similar
redox potential as, and can displace, iron in hemoglobin or an iron atom-dependent
dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of
an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron
atom-dependent dioxygenase, a compound or complex containing said transition metal or
compound, hydralazine and a hydralazine-related compound said agent elevating levels of
EPCs or cells of EPC lineage and enhancing the angiogenic capability of said cells and/or
optionally mobilizing said cells to a site in the systemic vasculature.

The subject method may also be used to stabilize HIF protein. This has implications not
only for angiogenesis but a range of genes controlled by HIF. By targeting a master control
gene (e.g. HIF-1α) a better angiogenic response is expected than that which occurs when a
single protein targeted therapy is utilised. The invention is also likely to overcome issues
associated with the short half life of protein based therapeutics and drug delivery problems
associated with protein and gene therapy based approaches to angiogenic therapies.
Further, since HIF-1 controls endothelial cell secretion of stromal cell derived factor-1
(SDF-1) and SDF-1 has been shown to be upregulated in ischemic myocardium and to
enhance stem cell engraftment in a model of ischemic cardiomyopathy, it is anticipated
that HIF-1 stabilisation with the compounds described herein will increase EPC cell
numbers and allow for targeting of these cells to areas expressing SDF-1 as induced by the
compounds that are the subject of this patent resulting in a decrease in overall
cardiovascular risk (Ceradini et al, Nature Medicine, 10: 858-864, 2004; Askari et al,

Hence, another aspect is directed to a method for stabilizing a HIF protein in a cell of a
subject said method comprising administering to said subject a HIF protein stabilizing effective amount of an agent selected from a transition metal or compound which has the same or similar redox potential as, and can displace, iron in hemoglobin or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound.

Yet a further aspect provides a method for promoting angiogenesis or vasculogenesis in a subject said method comprising administering to said subject an effective amount of an agent or combination of agents which elevate levels of stem cells or precursor cells thereof or early committed cells of a particular lineage and which enhance angiogenic or vasculogenic capability of said cells.

Again, particularly, the agents also mobilize the cells to a site in the systemic vasculature.

Still another aspect contemplates the use of an agent which elevates levels of stem cells, precursor cells thereof or early committed cells of a particular cell lineage and which enhances the angiogenic or vasculogenic capability of said cells and/or which optionally mobilizes said cells to a site within the systemic vasculature in the manufacture of a medicament for the treatment of an event, condition and/or disease of the systemic vasculature.

Another aspect provides a method for increasing erythropoietin production comprising administering to said subject an agent selected from a transition metal or compound which has the same or similar redox potential as, and can displace, iron in hemoglobin or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound, for use in the treatment of anemia such as but not limited to anemia associated with chronic renal
failure, heart failure or anemia of chronic disease.

A particular embodiment provides for the use of cobalt, a compound comprising cobalt (e.g. hydroxycobalamin [Vitamin B12]), hydralazine or a hydralazine-related compound in the manufacture of a medicament for the treatment of an event, condition or disease of the systemic vasculature such as but not limited to CAD, cardiac hypertrophy, ischemic stroke, anemia, ulcer or wound and/or PAD.

Further contemplated is a method for the treatment or prophylaxis of infection by a pathogenic agent said method comprising the administration of an agent selected from a transition metal or compound which has the same or similar redox potential as, and can displace, iron in hemoglobin or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound, to elevate or stabilize HIF-1.

As indicated above, also included are pharmaceutical compositions and formulations which comprise the instant agents. The pharmaceutical compositions herein may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including cardiac patches, wound dressings, creams, lotions etc), pulmonary, (e.g. by inhalation or insufflation of powders or aerosols, including by nebulizer, intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, perivascular, intraarterial, intracoronary injection into the heart or peripheral heart muscle, intrapericardial delivery, intraperitoneal or intramuscular injection or infusion; or intracranial, (e.g. intrathecal or intraventricular) administration, retroinfusion into the myocardium via a coronary vein, patches, catheters, NOGA catheter for intramyocardial injection, stents and other specialized drug delivery devices are also contemplated by the present invention.
The pharmaceutical formulations, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions herein may be formulated into any of many possible dosage forms such as, but not limited to injectable solutions or liquids, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

Compositions herein include, but are not limited to, solutions, emulsions and foams. The compositions and formulations herein may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients.

Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. Emulsions may contain additional components in addition to the dispersed phases, and the active drug which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Microemulsions are encompassed herein. Emulsions and their uses are well known in the art and are further described in U.S. Patent 6,287,860, which is incorporated herein in its entirety.

The active agents of the present invention may be in crystalline form, either as the free compounds or as solvates (e.g. hydrates). Methods of solvation are generally known within the art.
The salts of the active compounds are preferably pharmaceutically acceptable, but it will be appreciated that non-pharmaceutically acceptable salts also fall within the scope of the present invention, since these are useful as intermediates in the preparation of pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts include salts of pharmaceutically acceptable cations such as sodium, potassium, lithium, calcium, magnesium, ammonium and alkylammonium; acid addition salts of pharmaceutically acceptable inorganic acids such as hydrochloric, orthophosphoric, sulfuric, phosphoric, nitric, carbonic, boric, sulfamic and hydrobromic acids; or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, trihalomethanesulphonic, toluenesulphonic, benzenesulphonic, salicylic, sulphanilic, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothentic, tannic, ascorbic and valeric acids.

The term "pro-drug" is used herein in its broadest sense to include those compounds which can be converted in vivo to the compound of interest (e.g. by enzymatic or hydrolytic cleavage). Examples thereof include esters, such as acetates of hydroxy or thio groups, as well as phosphates and sulphonates. Processes for acylating hydroxy or thio groups are known in the art, e.g. by reacting an alcohol (hydroxy group), or thio group, with a carboxylic acid. Other examples of suitable pro-drugs are described in Bundgaard Design of Prodrugs, Elsevier 1985, the disclosure of which is included herein in its entirety by way of reference.

The term “metabolite” includes any compound into which the active agents can be converted in vivo once administered to the subject. Examples of such metabolites are glucuronides, sulphates and hydroxylates.

It will be understood that active agents as described herein may exist in tautomeric forms.

The term "tautomer" is used herein in its broadest sense to include compounds capable of existing in a state of equilibrium between two isomeric forms. Such compounds may
differ in the bond connecting two atoms or groups and the position of these atoms or
groups in the compound. A specific example is keto-enol tautomerism.

The compounds herein may be electrically neutral or may take the form of polycations,
having associated anions for electrical neutrality. Suitable associated anions include
sulfate, tartrate, citrate, chloride, nitrate, nitrite, phosphate, perchlorate, halosulfonate or
trihalomethylsulfonate.

The formulation of therapeutic compositions and their subsequent administration (dosing)
is believed to be within the skill of those in the art. Dosing is dependent on severity and
responsiveness of the disease state to be treated, with the course of treatment lasting from
several days to several months, or until a cure is effected or a diminution of the disease
state is achieved. Optimal dosing schedules can be calculated from measurements of drug
accumulation in the body of the patient. Persons of ordinary skill can easily determine
optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary
depending on the relative potency of the agents, and can generally be estimated based on
EC₅₀s found to be effective in in vitro and in vivo animal models. In general, dosage is
from 10μM to about 2000μM such as 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130,
140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310,
320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490,
500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670,
680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850,
860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000μM or 1100,
1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900 or 2000μM. This may be bolus or per
100kg of body weight. Persons of ordinary skill in the art can easily estimate repetition
rates for dosing based on measured residence times and concentrations of the drug in
bodily fluids or tissues. Following successful treatment, it may be desirable to have the
patient undergo maintenance therapy to prevent the recurrence of the disease state.

The compounds herein may be administered in a sustained (i.e. controlled) or slow release
form. A sustained release preparation is one in which the active ingredient is slowly
released within the body of the subject once administered and maintains the desired drug concentration over a minimum period of time. The preparation of sustained release formulations is well understood by persons skilled in the art. Dosage forms may include oral forms, implants and transdermal forms, sustained or slow release injectables and cardiac patches. For slow release administration, the active ingredients may be suspended as slow release particles or, for example, within liposomes, for example.

The compositions herein may be packaged for sale with other active agents or alternatively, other active agents may be formulated with the transition metal or hydralazine or their pharmaceutical salts, derivatives, homologs or analogs thereof. the term "pharmaceutical composition" may also be used to define the "composition".

Thus, a further particular aspect of the present invention provides a system for the controlled release of one or more agents selected from the list consisting of a transition metal or compound which has the same or similar redox potential as, and can displace, iron in hemoglobin and/or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound in combination with a pharmaceutically acceptable salt, derivative, homolog or analog thereof, alone or together with another active agent, wherein the system comprises:

(a) a deposit-core comprising an effective amount of a first active substance and having defined geometric form, and

(b) a support-platform applied to the deposit-core, wherein the support-platform contains a second active substance, and at least one compound selected from the group consisting of:

(i) a polymeric material which swells on contact with water or aqueous liquids and a gellable polymeric material wherein the ratio of the swellable polymeric material to the gellable polymeric material is in the range 1:9 to 9:1, and

(ii) a single polymeric material having both swelling and gelling
properties, and wherein the support-platform is an elastic support applied to the
deposit-core so that it partially covers the surface of the deposit-core and follows
changes due to hydration of the deposit-core and is slowly soluble and/or slowly
gellable in aqueous fluids.

5

As used herein, the first active substance is preferably a transition metal such as cobalt,
nickel or manganese or hydralazine. A second active substance, if present, may include an
angiogenesis-facilitating agent and/or an analgesic.

10 The support-platform may comprise polymers such as hydroxypropylmethylcellulose,
plasticizers such as a glyceride, binders such as polyvinylpyrrolidone, hydrophilic agents
such as lactose and silica, and/or hydrophobic agents such as magnesium stearate and
glycerides. The polymer(s) typically make up 30 to 90% by weight of the support-
platform, for example about 35 to 40%. Plasticizer may make up at least 2% by weight of
the support platform, for example about 15 to 20%. Binder(s), hydrophilic agent(s) and
hydrophobic agent(s) typically total up to about 50% by weight of the support platform, for
example about 40 to 50%.

The tablet coating may contain one or more water insoluble or poorly soluble hydrophobic
excipients. Such excipients may be selected from any of the known hydrophobic cellulose
derivatives and polymers including alkylcellulose, e.g. ethylcellulose, hydroxypropyl
cellulose, hydroxypropylmethyl cellulose, carboxymethyl cellulose, and derivatives
thereof; polymethacrylic polymers, polyvinyl acetate and cellulose acetate polymers; fatty
acids or their esters or salts; long chain fatty alcohols; polyoxyethylene alkyl ethers;
polyoxyethylene stearates; sugar esters; lauroyl macrogol-32 glyceryl, stearoyl macrogol-
32 glyceryl, and the like. Hydroxypropylmethyl cellulose materials are preferably selected
from those low Mw and low viscosity materials such as E-Type methocel, and 29-10 types
as defined in the USP.

30 Other agents or excipients that provide hydrophobic quality to coatings may be selected
from any waxy substance known for use as tablet excipients. Suitable hydrophobic agents
include waxy substances such as carnauba wax, paraffin, microcrystalline wax, beeswax, cetyl ester wax and the like; or non-fatty hydrophobic substances such as calcium phosphate salts, e.g. dibasic calcium phosphate.

Particularly the coating contains a calcium phosphate salt, glyceryl behenate, and polyvinyl pyrrolidone, or mixtures thereof, and one or more adjuvants, diluents, lubricants or fillers.

Particular components in the coating are as follows, with generally suitable percentage amounts expressed as percentage weight of the coating.

Polyvinyl pyrrolidone (Povidone) is preferably present in amounts of about 1 to 25% by weight or the coating, more particularly 4 to 12%, e.g. 6 to 8%.

Glyceryl behenate is an ester of glycerol and behenic acid (a C22 fatty acid). Glyceryl behenate may be present as its mono-, di-, or tri-ester form, or a mixture thereof. Preferably it has an HLB value of less than 5, more preferably approximately 2. It may be present in amounts of about 5 to 85% by weight of the coating, more particularly from 10 to 70% by weight, and in certain preferred embodiments from 30 to 50%.

Calcium phosphate salt may be the dibasic calcium phosphate dihydrate and may be present in an amount of about 10 to 90% by weight of the coating, preferably 20 to 80%, e.g. 40 to 75%.

The coating may contain other common tablet excipients such as lubricants, colorants, binders, diluents, glidants and taste-masking agents or flavorants.

Examples of excipients include colorants such a ferric oxide, e.g. yellow ferric oxide; lubricants such as magnesium stearate; and glidants such as silicon dioxide, e.g. colloidal silicon dioxide. Yellow ferric oxide may be used in amounts of about 0.01 to 0.5% by weight based on the coating; magnesium stearate may be present in amounts of 1 to 20% by weight of the coating, more preferably 2 to 10%, e.g. 0.5 to 1.0%; and colloidal silica
may be used in amounts of 0.1 to 20% by weight of the coating, preferably 1 to 10%, more preferably 0.25 to 1.0%.

The core comprises in addition to a drug substance, a disintegrating agent or mixtures of disintegrating agents used in immediate release formulations and well known to persons skilled in the art. The disintegrating agents useful in the exercise of the present invention may be materials that effervesce and or swell in the presence of aqueous media thereby to provide a force necessary to mechanically disrupt the coating material.

Preferably a core contains, in addition to the drug substance, cross-linked polyvinyl pyrrolidone and croscarmellose sodium.

The following is a list of preferred core materials. The amounts are expressed in terms of percentage by weight based on the weight of the core.

Cross-linked polyvinyl pyrrolidone is described above and is useful as a disintegrating agent, and may be employed in the core in the amounts disclosed in relation to the core.

Croscarmellose sodium is an internally cross-linked sodium carboxymethyl cellulose (also known as Ac-Di-Sol) useful as a disintegrating agent.

Disintegrating agents may be used in amounts of 5 to 30% by weight based on the core. However, higher amounts of certain disintegrants can swell to form matrices that may modulate the release of the drug substance. Accordingly, particularly when rapid release is required after the lag time it is preferred that the disintegrants is employed in amounts of up to 10% by weight, e.g. about 5 to 10% by weight.

The core may additionally comprise common tablet excipients such as those described above in relation to the coating material. Suitable excipients include lubricants, diluents and fillers, including but not limited to lactose (for example the mono-hydrate), ferric oxide, magnesium stearates and colloidal silica.
Lactose monohydrate is a disaccharide consisting of one glucose and one galactose moiety. It may act as a filler or diluent in the tablets of the present invention. It may be present in a range of about 10 to 90%, preferably from 20 to 80%, and in certain preferred embodiments from 65 to 70%.

As stated above, it is an important aspect of the present invention that core is correctly located within the coating to ensure that a tablet has the appropriate coating thickness.

Details of pharmaceutically acceptable carriers, diluents and excipients and methods of preparing pharmaceutical compositions and formulations are provided in Remmington's *Pharmaceutical Sciences* 18th Edition, 1990, Mack Publishing Co., Easton, Pennsylvania, USA.

Further provided are mechanical devices for introduction to or in a body or body cavity coated with a sustained or slow release formulation of one or more agents selected from the list consisting of a transition metal or compound which has the same or similar redox potential as, and can displace, iron in hemoglobin or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound. Examples of mechanical devices include stents, catheters, pins, needles, cardiac or arterial implants and the like. Reference to an "intradthecal implant" includes reference to a cylindrical thread or device comprising a semipermeable membrane which permits passage or partial passage of small molecules (such as nutrients ad drugs in and cellular metabolic products out). The implant may also contain genetically modified or cultured cells (including stem cells such as EPCs) which secrete out useful cytokines and other metabolites. The implant may be designed to release molecules (or intake cellular by-products) for days, weeks, months or even years.

Stents, for example, typically have a lumen, inner and outer surfaces, and openings
extending from the outer surface to the inner surface. The present invention extends to a method for coating a surface of a stent. At least a portion of the stent is placed in contact with a coating solution containing a coating material to be deposited on the surface of the stent. A thread is inserted through the lumen of the stent, and relative motion between the stent and the thread is produced to substantially remove coating material within the openings.

The thread can have a diameter substantially smaller than the diameter of the lumen. The thread can be inserted through the lumen either after or prior to contacting the stent with the coating solution. Relative motion between the stent and the thread can be produced prior to contacting the stent with the coating solution to clean the stent. The thread can be either a filament or a cable with a plurality of wires. The thread can be made of a metallic or polymeric material.

The stent can be dipped into the coating solution or spray coated with the coating solution. The coating material can include a biocompatible polymer, either with or without a pharmaceutically active compound.

In one embodiment, the relative motion is oscillatory motion produced by a vibrating device. The oscillations can be changed (magnitude and/or frequency) to vary thickness of the coating solution on the stent. In another embodiment, the relative motion is produced by a shaker table. Regardless of the type of motion, the relative motion can be produced either after or while the stent is in contact with the coating solution.

The relative motion between the stent and the thread can include initially moving the stent in a horizontal direction substantially parallel to the length of the thread and subsequently moving the stent in a vertical direction substantially perpendicular to the length of the thread. The movement in the horizontal direction can be repeated, with pauses between repetitions. The movement in the vertical direction can also be repeated, with the horizontal and vertical movements alternating.
In order to smooth the relative motion, the thread can be coupled to a damping compensator. The damping compensator connects the thread to a vibrating device. In one embodiment, the damping compensator comprises first and second filaments connected to the thread.

The relative motion can be motion of the stent along the thread. For example, a first end of the thread can be attached to a first stand at a first height and a second end of the thread is attached to a second stand at a second height. The relative motion is produced by a gravity gradient, with the first height differing from the second height. Furthermore, the stent can be moved back and forth between the first and second stands by sequentially increasing or decreasing at least one of the first and second heights. In this way, multiple coatings can be applied to the stent.

The relative motion can also be rotation of the stent relative to the thread. A stream of gas can be passed along at least a portion of the surface of the stent to rotate the stent relative to the thread. The rotation can also occur in conjunction with other relative motion between the stent and the thread.

Further provided is an implantable medical device having an outer surface covered at least in part by an agent selected from the list consisting of a transition metal or compound which has the same or similar redox potential as, and can displace, iron in hemoglobin and/or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound or a pharmaceutically acceptable salts, derivative, homolog or analog thereof or other active agent, a conformal coating of a hydrophobic elastomeric material incorporating an amount of active material therein for timed delivery therefrom and means associated with the conformal coating to provide a non-thrombogenic surface after the timed delivery of the active material.
Particularly, the conformal coating comprises an amount of finely divided biologically active material in the hydrophobic elastomeric material.

The implantable medical device includes a catheter.

Also provided are the use of functional foods in the treatment of events, conditions and/or diseases of the systemic vasculature. A "functional food" contains a transitional metal (e.g. cobalt, nickel or manganese) or hydralazine in an amount effective to enhance levels of EPCs or other stem cells and enhance their angiogenic properties. The agents may be naturally occurring in the food or may be a dietary supplement. Hence, dietary therapeutic protocols are proposed in the promotion of angiogenesis.

The present invention may also be used in conjunction with diagnostic protocols. Hence, upon detection of certain parameters indicative of an event, condition or disease of the systemic vasculature, appropriate therapy (e.g. administration of the subject agent) may occur.

Examples of parameters include biological parameters such as one or more of myoglobin, myosin light chain (MLC), myosin heavy chain (MHC), total creatine kinase (CK) including CK-MB, lactate dehydrogenase (LDH-H4), aspartate aminotransferase (AST), cardiac troponin I and T (cTn-I and cTn-T, respectively) and cTn-I and cTn-I RNA, fatty acid binding protein (FAB protein) including FABP1 and human heart-type, glycogen phosphorylase-BB isoenzyme, α-atrial natriuretic peptide (ANP), cytoplasmic FABP, brain natriuretic peptide (BNP), adrenomedullin (ADM), low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL) and intermediate density lipoprotein (IDL), C reactive protein (CRP), serum amyloid A, P-selectin, prostaglandins, platelet-activating factor (PAF), histamine, tumor necrosis factor α (TNFα), soluble TNF receptor 2 (sTNFR2), fibrin, fibrinogen, fibronolytic peptides, modified haemoglobin (HbA1c), ferritin, soluble intercellular adhesion molecule (ICAM) including soluble intercellular adhesion molecule-1 (ICAM1), heat shock proteins, apoB, apoA, apoE, homocysteine or parts thereof, Streptococcus sp., Porphyromonas gingivalis, Helicobacter
pylori and Chlamydia pneumoniae or immunological relatives thereof, necrosis and
platelet markers, leptin, vasopeptidase inhibitor of cardiac endogenous kinins, heparin,
metalloproteinase-9, metalloproteinase-1 including its tissue inhibitor, angiotensin-
converting enzyme, CD95/Apo1/Fas, hepatocyte growth factor, soluble vascular cell
adhesion molecule-1 (VCAM1), plasma brain natriuretic peptide, angiotensin II type
receptor, endothelial constitutive nitric oxide synthase, glycoprotein IIIa genetic
polymorphisms, factor VIIa, thrombin, endothelin-1, cardiac myofibrillar proteins, Fas and
Fas ligand, ligands thereof or binding partners thereof or nucleic acid molecules encoding
same or their fragments or ligands or binding partners.

The present invention will now be further described with reference to the following
examples, which are intended for the purpose of illustration only and are not intended to
limit the generality of the subject invention as hereinbefore described.
EXAMPLE 1

*Measuring HIF-1 Levels Using Western Blotting*

HIF-1 protein levels are measured using Western Blotting (Towbin *et al.*, *Proc Natl Acad Sci*, 76:4350-4354, 1979; Burnette, *Anal Biochem*, 112:195-203, 1981; Towbin and Gordon, *J Immunol Methods*, 72:313-340, 1984). Briefly, electrophoretic separation of a protein lysate is carried out under denaturing conditions. Electrophoretically separated protein lysate components are transferred from a gel to a solid support and probed with an antibody that is specific for amino acids of the HIF-1 molecule. The antibodies react specifically with antigenic epitopes displayed by HIF-1 attached to a solid support.

EXAMPLE 2

*Assessing HIF-1 DNA-Binding Activity with Electrophoretic Mobility Shift Assay*

The DNA-binding activity of HIF-1 is assessed using Electrophoeretic Mobility Shift Assays as outlined in Maloyan *et al.*, *Physiol Genomics*, 23(1):79-88, 2005. DNA complexes migrate more slowly than unbound radioactive probe and are thus visualized by discrete bands of radioactivity near the top of an acrylamide gel image.

EXAMPLE 3

*Evaluation of Expression of Genes Controlled by HIF-1 by RT-PCR*

EXAMPLE 4

*Evaluation of EPC Levels*

The quantification of EPCs is performed by counting the number of cells that are positive for both acLDL uptake and isolectin-binding and which are adherent to fibronectin or by counting the number of colony forming units that are positive for both acLDL uptake and isolectin-binding. Alternatively, flow cytometric analysis using any combination of markers such as CD34, AC133 and KDR (kinase inert domain containing receptor) is undertaken on unfractionated bone marrow, peripheral blood cells, purified mononuclear cells and/or cultured mononuclear cells (Liew et al, Bioessays 28(3):261-270, 2006). However, it is important to note that absolute agreement on the identification of EPCs has not been reached (Khakoo AY, Finkel T. Endothelial progenitor cells. Annu Rev Med 2005;56:79-101.). These cells can contribute to angiogenesis or post-natal vasculogenesis in ischemic diseases.

EXAMPLE 5

*Animal Models of Atherosclerosis and Ischemia*

The following animal models are used to test the effects of cobalt containing compounds and/or hydralazine.

**Model 1: Ischemia of the Lower Limb**

The hindlimb ischemia model provides a model for the study of Peripheral Arterial Disease and various models have utilized rats or rabbits. Briefly, Sprague-Dawley rats with an average weight of approximately 360g are anaesthetized and treated with an intraperitoneal injection of 40 mg/kg ketamine and 5 mg/kg xylazine mixture. An additional injection of the mixture is given as needed. The animals are allowed to breathe a gas mixture of 95% v/v oxygen and 5% v/v carbon dioxide through a tight fitting mask and their body temperatures are kept at 37°C with a heating pad. All animals receive post-operative cefazolin (25 mg/kg) intramuscularly. Lower limb ischemia is produced by excising the superficial femoral artery after ligating the external iliac artery at the inguinal ligament and
the femoral artery at the point where the latter bifurcates to from the poplateral and saphenous branch arteries.

One assay is to assess skeletal muscle angiogenesis by capillary density and capillary per muscle fiber ratio count. At various time points, the animals are sacrificed with sodium pentobarbital and muscle samples from the middle portion of every leg muscle in the lower limb are removed. The samples are frozen in isopentane (pre-cooled at -70°C) and sectioned using a cryostat transversely at a thickness of 10µm. The sections are fixed in acetone (4°C, 5min) followed by 1 h incubation at 38°C in medium containing 30mg nitroblue tetrazolium and 2 mg 5-bromo-4-chloro-indolyl phosphate p-toluidine salt (dissolved in 30ml of buffer containing 6.9 mmol/liter MgSO₄, 27.5 mmol/liter NaBO₂, pH 9.3). The slides are then rinsed in aliquots of the above buffer, post fixed in sucrose-buffered formalin and counterstained in 0.5% w/v eosin. The sections are dehydrated, cleared and mounted with the use of alcohol, xylene substitute and mounting medium. For each sample six sections are prepared and six fields randomly selected and the capillary density and capillary per muscle fiber ratio determined by a microscopist. Statistical analysis is undertaken to analyze differences between groups on capillary density and capillary per muscle fiber ratio.

Using this model the effects of treatment with cobalt and hydralazine before during or after ischemia of the lower limb is assessed using angiographic and hemodynamic measures and by the assessment of adductor muscle blood flow. The efficacy of administration of cobalt and/or hydralazine is shown by improved angiographic and hemodynamic measures and increased adductor muscle blood flow. The levels of HIF-1 protein and gene expression and EPC numbers are also assessed using the techniques outlined in Examples 1 and 4 above. The effects of administration of cobalt and/or hydralazine prior to, during or following ischemia of the lower limb is shown by an increase in HIF-1 protein and/or a stabilizing of levels of HIF-1 protein as well as gene expression levels of at least angiogenesis-associated genes, an increase in EPC numbers and/or an increase in capillary density and capillary per muscle fiber ratio (Stark et al. Journal of Surgical Research, 79: 8-12, 1998).
Model 2: Myocardial Ischemia

*In vivo* studies are undertaken using models of myocardial ischemia and coronary arterial thrombosis, coronary arterial occlusions, stunning, myocardial hibernation and ischemic preconditioning as outlined in Verdouw et al, *Cardiovascular Research*, 39:121-135, 1998; in dog, sheep, marmot, ferrets, baboon, guinea pig, pig, cat, rabbit and rat. Further studies are undertaken using isolated cardiac myocytes and isolated perfused hearts. The levels of HIF-1 protein and gene expression and EPC numbers are also assessed using the techniques outlined in Examples 1 and 4 above. The efficacy of the administration of cobalt and/or hydralazine prior to, during or following ischemia, thrombosis, occlusions, stunning and/or myocardial hibernation is shown by an increase in HIF-1 protein and possibly gene expression levels and an increase in EPC numbers. This translates into a reduced infarct size as assessed by cardiac magnetic resonance imaging, histology or echocardiography and reduced left ventricular remodelling as determined by left ventricular ejection fraction, myocardial contractility and ventricular dilatation (Hossmann, *Cardiovascular Research*, 39:106-120, 1998 and Trastman, *Institute of Laboratory Animal Resources Journal*, 44:85-95, 2003).

Model 3: Coronary Artery Ligation Model of Heart Failure

The coronary artery ligation model in rats and/or mice is also utilised as outlined in (Goldman and Thomas, *Journal of Cardiac Failure*, 1 169-177, 1995). The model involves mechanical ventilation of the animal and thoracotomy, after which a coronary artery (usually the left coronary or branch) is occluded causing acute ischemia and myocardial infarction.

Briefly, after induction of anesthesia in adult Sprague-Dawley rats, Fischer 344 and Brown Norway Fischer 344 cross rats with acepromazine maleate (50mg/kg), xylazine (5mg/kg) and ketamine HCl (50 mg/kg) intraperitoneally, a (left) anterior thoracotomy is performed under sterile conditions. The heart is expressed through the incision and a 7-0 synthetic ligature is secured snugly around the proximal left anterior coronary artery. The lungs are inflated to reduce the pneumothorax and the muscle layer and skin are closed separately.
Postoperative analgesia is provided with acetaminophen (67 mg/L) in the drinking water. Other variations on this basic approach are to use endotracheal intubation with ventilator support so as to have more time to perform the ligation and to treat rats with perioperative lidocaine to decrease the incidence of ventricular tachycardia and fibrillation.

Electrocardiographic (ECG) screening may also be used to stratify rats with respect to the size of the infarction. To obtain an ECG, rats are anaesthetized with a short-acting inhaled anaesthetic and a nine-lead ECG with six limb leads and three chest leads is performed. The rats with evidence of large myocardial infarctions can be selected with high specificity using ECG criteria for a large infarction including the presence of Q waves (>1 mV) in the limb leads (I or a VL) and the sum of R waves in the precordial leads less than 10 mV. The additional criteria of absence of an R wave in leads I and aVL are added, the specificity for large myocardial infarction is greater than 97%. The infarct scar size and/or the volume of infarcted and noninfarcted tissue is also quantified after the animal is sacrificed and the heart is excised (Goldman and Thomas, 1995 supra). Larger animal models are also utilized and the preferred is the pig model because they have fewer collaterals than dogs. Also, an ameroid constrictor can be placed around the proximal left circumflex artery to produce progressive stenosis over a 2-4 week period (Ruel et al, Mol Cell Biochem, 264:119-31, 2004).

The levels of HIF-1 protein and gene expression and EPC numbers are also assessed using the techniques outlined in Examples 1 and 4 above. The increase in HIF-1 protein and gene expression levels and an increase in EPC numbers administration of a cobalt containing compound and/or hydralazine containing compound prior to, during or following coronary artery ligation. The outcomes upon administration of a cobalt containing compound and/or hydralazine containing compound are outlined in model 2 supra.

**Model 4: Cerebral Ischemia Models**

The focal cerebral ischemia animal models including models of intracranial vascular occlusion, microcirculatory occlusion, extracranial vascular occlusion, occlusion of the common carotid artery, occlusion of anterior cerebral artery, occlusion of the middle

The models of global brain ischemia, complete and incomplete brain ischemia, microembolism and in vitro ischemia including models of cardiac arrest by ventricular fibrillation, intrathoracic hook, induced hypertension, occlusion of the aorta, occlusion of arteria pulmonalis, occlusion of the vena cava, pneumatic cuff around the neck, occlusion of carotid arteries, hypotension, hypoxia, occlusion of carotid and vertebral arteries, occlusion of innominate and subclavian arteries, intracranial hypertension, isolated head, decapitation, in vitro neuronal cultures, in vitro organotypic culture and in vitro brain slices are utilised as outlined in Hossmann, 1998 supra and Traystman, 2003 supra. As above, infarct size is assessed with magnetic resonance imaging.

Model 5: Carotid Artery Denudation

The Carotid Artery Denudation model provides a model for the study of stroke related carotid atherosclerosis. An arteriotomy is made in the right common artery in rabbits after this vessel has been clamped and a 3-French Fogarty balloon catheter (Baxter Healthcare Corp) is introduced into the lumen. Arteries undergo endothelial denudation by passage of a deflated balloon catheter three times. The administration of cobalt and/or hydralazine before, during or after the carotid artery denudation enhances endothelialization as determined by Evans Blue staining (He et al, Stroke, 35: 2378-2384, 2004).
EXAMPLE 6

The Preparation of Stem Cells For Cardiovascular Grafts

Stem cells are isolated and seeded in three-dimensional matrices of polymers to form 3-D living tissue products having structural or functional properties that can be used to restore, maintain or improve tissue function as outlined in Wu et al, Transplant Immunology, 16: 1-7, 2006.

Coating three-dimensional matrices of polymers or any substances to be placed in the human body with cobalt and/or hydralazine is expected to attract stem cells and to enhance the incorporation of grafts into the body. One example is the use of cobalt/hydralazine to increase EPC numbers to enhance the endothelial coating of synthetic grafts such as Dacron used in a vascular setting. Cobalt and/or hydralazine are also utilized to enhance stem cell recruitment in vivo to enhance blood flow to a explant when a scaffold is being used for tissue engineering (for example moulds in the peritoneal cavity for vessel and valves) [Wu et al,2006 supra].

EXAMPLE 7

Enhancing Circulating Stem Cell Numbers For Cardiovascular Risk Reduction or Tissue/Organ Engineering

Hydralazine and/or cobalt containing compounds are administered using topical (including cardiac patches, wound dressings, creams, lotions etc), pulmonary, (e.g. by inhalation or insufflation of powders or aerosols, including by nebulizer, intratracheal, intranasal, epidermal and transdermal), oral or parenteral methods. Parenteral administration includes intravenous, perivascular, intraarterial, intracoronary injection into the heart or peripheral heart muscle, intrapericardial delivery, intraperitoneal or intramuscular injection or infusion; or intracranial, (e.g. intrathecal or intraventricular) administration, retroinfusion into the myocardium via a coronary vein, patches, catheters, NOGA catheter for intramyocardial injection, stents and other specialized drug delivery devices, to enhance circulating stem cell numbers, which are then harvested from a peripheral vein and
cultured \textit{in vitro} using the methods outlined in Wu \textit{et al}, 2006 \textit{supra}. EPC cells numbers are evaluated as outlined in Example 4 above. This example pertains to the harvest of stem cells to produce artificial organs \textit{in vitro} or to enhance \textit{in vivo} maturation of organs that have been developed \textit{in vitro}. HIF-1 regulates the expression of stromal derived factor-1 (SDF-1) which is the ligand for CXCR4 which is expressed on numerous putative stem cells and progenitor cells (Ceradini \textit{et al.} \textit{Nature Medicine}, 10: 858-64, 2004). Hence, cobalt and/or hydralazine may be used to locally induce expression of SDF-1 by endothelium and thereby attract numerous stem cell types to an area of interest.

\textbf{EXAMPLE 8}

\textit{Enhancing Wound Healing}

Small mammal and pig models are utilized for wound healing studies as outlined in Sullivan \textit{et al}, \textit{Wound Repair and Regeneration}, 9:66-76, 2001, Wanda \textit{et al}, \textit{Wound Repair and Regeneration}, 12:591-599, 2004 and Greenhalgh, \textit{Journal of Burn Care and Rehabilitation}, 26:293-305, 2005. Enhanced wound healing is demonstrated following topical administration of cobalt and/or hydralazine in dressings or other applications is expected to enhance wound healing. Wound healing is assessed using histological assays such as the progression of new epithelium, inflammation vascular response, thickness of skin layers and the formation of collagen in the wound defect, assessment of the rate of reepithelialization, assessment of the proliferative response of keratinocytes in wounds, biochemical and biomechanical measurements, measurements of wound healing markers, an assessment of cellular and immunologic responses (Sullivan \textit{et al}, 2001 \textit{supra}; Wanda \textit{et al}, 2004 \textit{supra}). \textit{In vitro} models of tissue repair are outlined in Greenhalgh, 2005 \textit{supra}. Models include, but are not limited to, exposing cells to elevated temperature and measuring the expression of stress related proteins, assessing the action of external factors on the stimulation of chemotaxis or proliferation, examining the production of extracellular matrix, various angiogenesis models, epithelialization models, models of cellular contraction and combined tissue co-culture (Greenhalgh, 2005 \textit{supra}). The outcomes include mechanical strength of the wound by testing breaking strength with a tensiometer, analysis for areas of re-epithelialization with grid techniques. Enhanced wound strength or
hastened re-epithelialization is demonstrated following administration of cobalt and/or hydralazine containing compound.

**EXAMPLE 9**

*Anemia Treatment*

Cobalt and/or hydralazine containing compounds increase erythropoietin production (Goldwasser et al, *Blood*, 13:55-60, 1958) and hence are used to treat anemia associated with, but not exclusive to, chronic renal failure, heart failure or anemia of chronic disease. A clinical trial with the various modes of administration of these compounds is required and involves the recruitment of anemic patients. Outcome measures include erythropoietin levels and hemoglobin and exercise capacity.

**EXAMPLE 10**

*Clinical Trials*

Clinical trial using cobalt containing compounds and/or hydralazine containing compounds are conducted using the following parameters:

<table>
<thead>
<tr>
<th>Patient Selection</th>
<th>End Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Patients who do not have a threatened obstruction or who require revascularization using conventional methods are preferably utilised.</td>
<td>• Exercise tolerance:</td>
</tr>
<tr>
<td>• Randomization should account for age, sex, cholesterol, hsCRP, native angiogenic response. These factors are recorded and balanced between groups.</td>
<td>• High variation in day to day variation in patients with CAD.</td>
</tr>
<tr>
<td>• The status of patients in relation to drugs that could decrease angiogenesis, such as non-steroidal anti-inflammatory drugs, cyclooxygenase 2 inhibitors, statins, NO donors and captopril is recorded and</td>
<td>• Validity is questionable as some agents such as Milrinone have beneficial effects on exercise time, but adversely affect mortality.</td>
</tr>
<tr>
<td></td>
<td>• Subjective indications are used for &quot;stopping test&quot;.</td>
</tr>
<tr>
<td></td>
<td>• Patients who show large variability on 2 consecutive tests are excluded.</td>
</tr>
<tr>
<td>balanced between study groups.</td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td></td>
</tr>
<tr>
<td>• A baseline cancer screen is undertaken in patients &gt;50 with no precancerous lesions in the previous 5 years, including: mammography, PSA, sigmoidoscopy.</td>
<td></td>
</tr>
<tr>
<td>• Patients with prior malignancies are excluded from the trial.</td>
<td></td>
</tr>
<tr>
<td>• Patients with proliferative retinopathies are excluded from the trial.</td>
<td></td>
</tr>
<tr>
<td>• Patients with renal impairment are excluded from the trial until the effect of cobalt on renal toxicity is established.</td>
<td></td>
</tr>
<tr>
<td>• Patients exhibiting angina at rest are excluded from the trial.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Death:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• A robust end point that requires longer follow up at a greater cost.</td>
</tr>
<tr>
<td>• Suitable for trials that include patients with ischemic heart failure who are not amenable to revascularization. Such patients have a higher mortality rate, increasing statistical significance and shortening follow-up.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Quality of Life:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Canadian Cardiovascular Society functional class is an outcome measure.</td>
</tr>
<tr>
<td>• Generic health status measures such as the Canadian Cardiovascular Society functional class are used as a secondary end point.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Myocardial Perfusion Scan:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• For use in patients enrolled in angiogenesis trials who have widespread vessel disease and have unsatisfactory results with exercise testing. In such cases it may be difficult to match exactly myocardial demand and blood pressure on repeat exercise testing. Pharmacologic stress testing with myocardial perfusion scan can be particularly useful since use of dipyridamole or adenosine is more reproducible.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Magnetic Resonance Imaging:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Magnetic Resonance Imaging with Gadolinium enhancement to detect improvements in blood flow, flow reserve and collateral flow.</td>
</tr>
<tr>
<td>Resolution allows for visualization of transmural gradients.</td>
</tr>
<tr>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Angiography:</strong></td>
</tr>
<tr>
<td>• The collaterals which are visualised depend on the injection technique.</td>
</tr>
<tr>
<td>• Limited spatial resolution</td>
</tr>
<tr>
<td>• Subjective assessment of collaterals.</td>
</tr>
</tbody>
</table>

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 of any two or more of said steps or features.
BIBLIOGRAPHY


American Heart Association, *Heart and Stroke Statistical Update*, 2002


Ceradini et al, *Nature Medicine, 10*: 858-64, 2004


Cooke and Losordo *Circulation 105*(18):2133-2135, 2002


Ghani et al, Stroke, 36: 151-153, 2005


Goldberg and Schneider, J Biol Chem 269(6):4355-9, 1994

Goldman and Thomas, Journal of Cardiac Failure, 1 169-177, 1995


Greenhalgh, Journal of Burn Care and Rehabilitation, 26:293-305, 2005


He et al, Arch Intern Med, 161:996-1002, 2001

He et al, Stroke, 35: 2378-2384, 2004

Heart Disease and Stroke Statistics Update, 2006


Hill et al, J Am Coll Cardiol, 46: 1643-1648, 2005


Hossmann, Cardiovascular Research, 39:106-120, 1998


Hunt, J Am Coll Cardiol, 46(6):e1-82, 2005


Jelkmann, Physiol Rev 72(2):449-89, 1992


Juan et al, Eur Heart J. 25(14):1197-1207, 2004


Katritsis et al, Circulation, 111: 2906-12, 2005

Khakoo et al, Bioessays, 28(3):261-70, 2006


Wanda et al, Wound Repair and Regeneration, 12:591-599, 2004


Wojakowski et al, Circulation, 110: 3213-3220, 2004


Wright et al, Gene Therapy, 8:1833-1839, 2001

Wu et al, Transplant Immunology, 16: 1-7, 2006


Yu et al, J Clin Invest 103(5):691-6, 1999


CLAIMS:

1. A method for the treatment or prophylaxis of an event, condition and/or disease of, or affecting, the systemic vasculature in a subject, said method comprising administering to said subject an effective amount of an agent or combination of agents which elevates levels of stem cells or precursor cells thereof or early committed cells of a particular lineage and enhance angiogenic or vasculogenic capability.

2. The method of Claim 1 wherein said agent also mobilizes said cells to a site in the systemic vasculature.

3. The method of Claim 1 or Claim 2 wherein said stem cells are endothelial precursor cells (EPCs) or precursor cells thereof or early committed cells in an EPC lineage.

4. The method of Claim 3 wherein said stem cells are EPCs.

5. The method of Claim 1 wherein said subject is a mammal.

6. The method of Claim 5 wherein said mammal is a human.

7. The method of Claim 1 wherein said agent is selected from the list consisting of a transition metal or compound which has the same or similar redox potential as, and can displace, iron in hemoglobin or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound.

8. The method of Claim 1 wherein said agent stabilizes or otherwise elevates or maintains levels of a HIF protein.
9. The method of Claim 8 wherein said HIF protein is HIF-1.

10. The method of Claim 8 wherein said HIF protein is HIF-1α.

11. The method of Claim 1 wherein said agent is cobalt or a pharmaceutically acceptable salt thereof or a compound containing cobalt.

12. The method of Claim 11 wherein said cobalt or cobalt-containing compound is selected from the list consisting of cobalt, cobalt (II) acetate, cobalt (II) acetate tetrahydrate, cobalt (II) acetylacetonate, cobalt (ii) acetylacetonate hydrate, cobalt (III) acetylacetonate, cobalt (III) benzoylacetonate, cobalt boride, cobalt (II) bromide, cobalt (II) bromide hydrate, cobalt (II) carbonate hydrate, cobalt (II) chloride, cobalt (II) chloride hydrate, cobalt (II) chloride hexahydrate, cobalt (II) 2-ethylhexanoate, cobalt (II) fluoride, cobalt (II) fluoride tetrahydrate, cobalt (III) fluoride, cobalt (II) 1,2,3,4,8,9,10,11,15,16,17,18,22,23,24,25-hexadecafluoro-29H,31H-phthalocyanine, cobalt (II) hexafluoracetetylacetonate hydrate, cobalt (II) hydroxide, cobalt (II) iodide, cobalt (II) 2,3-napthalocyanine, cobalt (II) nitrate hexahydrate, cobalt (II) oxalate dihydrate, cobalt (II) oxide, cobalt (II,III) oxide, cobalt (II) perchlorate hexahydrate, cobalt (II) phthalocyanine, cobalt (II) selenide, cobalt (III) sephulchrate trichloride, cobalt (III) sephulchrate tris (tetr phenylborate), cobalt (II) sulfate hydrate, cobalt (II) tetrafluoroborate hexahydrate, cobalt (II) tetra thio cyanato mercurate, cobalt (II) thiocyanate, cobalt (II) thiocyanate hydrate, cobalt tris (2,2,6,6-tetramethyl-3,5-heptanedioate), cobalt (II) tungstate hydrate and hydroxycobalamin (Vitamin B12).

13. The method of Claim 1 wherein said agent is manganese or nickel or pharmaceutically acceptable salts thereof or a compound containing manganese and/or nickel.

14. The method of Claim 13 wherein said manganese or manganese containing compound is selected from the list consisting of manganese, manganese(II) acetate,
- 65 -

manganese(II) acetate tetrahydrate, manganese(III) acetate dihydrate, manganese(II) acetylacetonate, manganese(III) acetylacetonate, manganese(II) bromide, manganese(II) bromide tetrahydrate, manganese carbonyl, manganese(II) chloride, manganese(II) chloride tetrahydrate, manganese(II) cyclohexanbutyrate, manganese dioxidero, manganese(II) fluoride, manganese(III) fluoride, manganese(II) hexafluoroacetylacetonate trihydrate, manganese(II) hydrogen phosphite, manganese(II) hypophosphite monohydrate, manganese(II) iodide, manganese(II) nitrate hydrate, manganese(II) nitrate, manganese(II) oxide, manganese(II, III) oxide, manganese (III) oxide, manganese(IV) oxide, manganese(IV) oxide, 50% w/w on activated carbon, manganese(II) perchlorate hexahydrate, manganese(II) phthalocyanine, manganese(III) phthalocyanine chloride, manganese sesquioxide, manganese(II) sulfate hydrate, manganese(II) sulfate monohydrate, manganese(II) sulfide, manganese(III) 5,10,15,20-tetra(4-pyridyl)-21 H ,23 H --porphine chloride tetrakis(methochloride), manganic acetylacetonate, manganocene and manganous acetylacetonate.

15. The method of Claim 13 wherein said nickel or nickel containing compound is selected from the list consisting of nickel, nickel(II) acetate tetrahydrate, nickel(II) acetylacetonate, nickel(II) bis(2,2,6,6-tetramethyl-3,5-heptanedionate), nickel boride, nickel(II) bromide, nickel(II) bromide hydrate, nickel(II) bromide ethylene glycol dimethyl ether complex, nickel(II) bromide 2-methoxyethyl ether complex, nickel(II) carbonate, basic, nickel(II) carbonate hydroxide tetrahydrate, nickel(II) chloride, nickel(II) chloride hexahydrate, nickel(II) chloride hydrate, nickel(II) cyclohexanbutyrate, nickel(II) 2-ethylhexanoate, nickel(II) fluoride, nickel(II) fluoride tetrahydrate, nickel(II) formate dihydrate, nickel(II) hexafluoroacetylacetonate hydrate, nickel(II) hydroxide, nickel(II) iodide, nickel(II) nitrate hexahydrate, nickel(II) 5,9,14,18,23,27,32,36-octabutoxy-2,3-naphthalocyanine, nickel(II) 1,4,8,11,15,18,22,25-octabutoxy-29H ,31 H --phthalocyanine, nickel(II) octanoate hydrate, nickel, ~60% w/w on kieselghur, nickel, ~65% w/w on silica/alumina, nickel(II) oxalate dihydrate, nickel(II) oxide, nickel(II) perchlorate hexahydrate, nickel peroxide hydrate, nickel phosphide, nickel(II) phthalocyanine, nickel(II) phthalocyaninnetetraculfonic acid, tetrasodium salt, nickel(II) sulfamate tetrahydrate, nickel(II) sulfate heptahydrate, nickel(II) sulfate hexahydrate, nickel sulfide,

16. The method of Claim 1 wherein said agent is hydralazine or hydralazine-related compounds.

17. The method of any one of Claims 11 to 16 wherein the effective amount of the agent is from about 10$\mu$M to about 2000$\mu$M.

18. The method of Claim 1 or 17 wherein the mode of administration is via topical, pulmonary, oral or parental administration.

19. The method of Claim 18 wherein parental administration is via intravenous, perivascular, intraarterial, intracoronary, intrapericardial, intraperitoneal, intracranial, intramyocardial, catheter or stent administration.

20. A method for stabilizing a HIF protein in a cell of a subject said method comprising administering to said subject a HIF protein stabilizing effective amount of an agent selected from a transition metal or compound which has the same or similar redox potential as, and can displace, iron in hemoglobin or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound.

21. The method of Claim 20 wherein said subject is a mammal.

22. The method of Claim 20 wherein said HIF protein is HIF-1.

23. The method of Claim 20 wherein said HIF protein is HIF-1$\alpha$. 
24. The method of Claims 20 wherein said agent is cobalt or a pharmaceutically acceptable salt thereof or a compound containing cobalt.

25. The method of Claim 24 wherein said cobalt or cobalt-containing compound is selected from the list consisting of cobalt, cobalt (II) acetate, cobalt (II) acetate tetrahydrate, cobalt (II) acetylacetonate, cobalt (ii) acetylacetonate hydrate, cobalt (III) acetylacetonate, cobalt (III) benzoylacetonate, cobalt boride, cobalt (II) bromide, cobalt (II) bromide hydrate, cobalt (II) carbonate hydrate, cobalt (II) chloride, cobalt (II) chloride hydrate, cobalt (II) chloride hexahydrate, cobalt (II) 2-ethylhexanoate, cobalt (II) fluoride, cobalt (II) fluoride tetrahydrate, cobalt (III) fluoride, cobalt (II) 1,2,3,4,8,9,10,11,15,16,17,18,22,23,24,25-hexadeca-fluoro-29H,31H-phetalocyanine, cobalt (II) hexafluoroacetylacetonate hydrate, cobalt (II) hydroxide, cobalt (II) iodide, cobalt (II) 2,3-napthalocyanine, cobalt (II) nitrate hexahydrate, cobalt (II) oxalate dihydrate, cobalt (II) oxide, cobalt (II,III) oxide, cobalt (II) perchlorate hexahydate, cobalt (II) phthalocyanine, cobalt (II) selenide, cobalt (III) sephulchrate trichloride, cobalt (III) sephulchrate tris (tetr phenyl borate), cobalt (II) sulfate hydrate, cobalt (II) tetrafluoroborate hexahydrate, cobalt (II) tetrathiocyanatomercurate, cobalt (II) thiocyanate, cobalt (II) thiocyanate hydrate, cobalt tris (2,2,6,6-tetramethyl-3,5-heptanedioate), cobalt (II) tungstate hydrate and hydroxycobalamin (Vitamin B12).

26. The method of Claim 20 wherein said agent is manganese or nickel or pharmaceutically acceptable salts thereof or a compound containing manganese and/or nickel.

27. The method of Claim 26 wherein said manganese or manganese containing compound is selected from the list consisting of manganese, manganese(II) acetate, manganese(II) acetate tetrahydrate, manganese(III) acetate dihydrate, manganese(II) acetylacetonate, manganese(III) acetylacetonate, manganese(II) bromide, manganese(II) bromide tetrahydrate, manganese carbonyl, manganese(II) chloride, manganese(II) chloride tetrahydrate, manganese(II) cyclohexanbutyrate, manganese dioxide,
manganese(II) fluoride, manganese(III) fluoride, manganese(II) hexafluoroacetylacetonate trihydrate, manganese(II) hydrogen phosphite, manganese(II) hypophosphite monohydrate, manganese(II) iodide, manganese(II) nitrate hydrate, manganese(II) nitrate, manganese(II) oxide, manganese(II, III) oxide, manganese (III) oxide, manganese(IV) oxide, manganese(IV) oxide, 50% w/w on activated carbon, manganese(II) perchlorate hexahydrate, manganese(II) phthalocyanine, manganese(III) phthalocyanine chloride, manganese sesquioxide, manganese(II) sulfate hydrate, manganese(II) sulfate monohydrate, manganese(II) sulfide, manganese(III) 5,10,15,20-tetra(4-pyridyl)-21 H₂₃ H₂₃porphine chloride tetrakis(methochloride), manganic acetylacetone, manganocene and manganous acetylacetone.

28. The method of Claim 26 wherein said nickel or nickel containing compound is selected from the list consisting of nickel, nickel(II) acetate tetrahydrate, nickel(II) acetylacetonate, nickel(II) bis(2,2,6,6-tetramethyl-3,5-heptanedionate), nickel boride, nickel(II) bromide, nickel(II) bromide hydrate, nickel(II) bromide ethylene glycol dimethyl ether complex, nickel(II) bromide 2-methoxyethyl ether complex, nickel(II) carbonate, basic, nickel(II) carbonate hydroxide tetrahydrate, nickel(II) chloride, nickel(II) chloride hexahydrate, nickel(II) chloride hydrate, nickel(II) cyclohexanecarboxylate, nickel(II) 2-ethylhexanoate, nickel(II) fluoride, nickel(II) fluoride tetrahydrate, nickel(II) formate dihydrate, nickel(II) hexafluoroacetylacetonate hydrate, nickel(II) hydroxide, nickel(II) iodide, nickel(II) nitrate hexahydrate, nickel(II) 5,9,14,18,23,27,32,36-octabutoxy-2,3-naphthalocyanine, nickel(II) 1,4,8,11,15,18,22,25-octabutoxy-29H,31 H₂₃phthalocyanine, nickel(II) octanoate hydrate, nickel, ~60% w/w on kieselguhr, nickel, ~65% w/w on silica/alumina, nickel(II) oxalate dihydrate, nickel(II) oxide, nickel(II) perchlorate hexahydrate, nickel peroxide hydrate, nickel phosphide, nickel(II) phthalocyanine, nickel(II) phthalocyaninetetraculfonic acid, tetrasodium salt, nickel(II) sulfamate tetrahydrate, nickel(II) sulfate heptahydrate, nickel(II) sulfate hexahydrate, nickel sulfide, nickel(II) 2,11,20,29-tetra- tert-butyl-2,3-naphthalocyanine, nickel(II) tetrafluoroborate, nickel(II) tetrakis(4-cumylphenoxy)phthalocyanine, nickel(II) 2,9,16,23-tetraphenoxy-29H,31 H₂₃phthalocyanine, nickel(II) titanate, nickel TPP and Ni(COD)₂.
29. The method of Claim 20 wherein said agent is hydralazine or hydralazine-related compounds.

30. The method of Claim 20 wherein the effective amount of the agent is from about 10μM to about 2000μM.

31. The method of Claim 20 or 30 wherein the mode of administration is via topical, pulmonary, oral or parental administration.

32. The method of Claim 31 wherein parental administration is via intravenous, perivascular, intraarterial, intracoronary, intrapericardial, intraperitoneal, intracranial, intramyocardial, catheter or stent administration.

33. Use of an agent which elevates levels of stem cells, precursor cells thereof or early committed cells of a particular cell lineage and which enhances the angiogenic or vasculogenic capability of said cells and/or which mobilizes said cells to a site within the systemic vasculature in the manufacture of a medicament for the treatment of an event, condition and/or disease of the systemic vasculature.

34. Use of Claim 33 wherein said stem cells are endothelial precursor cells (EPCs) or precursor cells thereof or early committed cells in an EPC lineage.

35. Use of Claim 34 wherein the stem cells are EPCs.

36. Use of Claim 33 wherein said subject is a mammal.

37. Use of Claim 36 wherein said mammal is a human.

38. Use of Claim 33 wherein the agent is selected from the list consisting of a transition metal or compound which has the same or similar redox potential as, and can displace, iron in hemoglobin or an iron atom-dependent dioxygenase by complexing with the organic
protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound.

39. Use of Claim 33 wherein said agent is cobalt or a pharmaceutically acceptable salt thereof or a compound containing cobalt.

40. Use of Claim 39 wherein said cobalt or cobalt-containing compound is selected from the list consisting of cobalt, cobalt (II) acetate, cobalt (II) acetate tetrahydrate, cobalt (II) acetylacetone, cobalt (ii) acetylacetone hydrate, cobalt (III) acetylacetone, cobalt (III) benzoylecetonate, cobalt boride, cobalt (II) bromide, cobalt (II) bromide hydrate, cobalt (II) carbonate hydrate, cobalt (II) chloride, cobalt (II) chloride hydrate, cobalt (II) chloride hexahydrate, cobalt (II) 2-ethylhexanoate, cobalt (II) fluoride, cobalt (II) fluoride tetrahydrate, cobalt (III) fluoride, cobalt (II) 1,2,3,4,8,9,10,11,15,16,17,18,22,23,24,25-hexadeca-fluoro-29H,31H-phthalocyanine, cobalt (II) hexafluoroacetylacetone hydrate, cobalt (II) hydroxide, cobalt (II) iodide, cobalt (II) 2,3-napthalocyanine, cobalt (II) nitrate hexahydrate, cobalt (II) oxalate dihydrate, cobalt (II) oxide, cobalt (II,III) oxide, cobalt (II) perchlorate hexahydrate, cobalt (II) phthalocyanine, cobalt (II) selenide, cobalt (III) sephulchrate trichloride, cobalt (III) sephulchrate tris (tetraphenylborate), cobalt (II) sulfate hydrate, cobalt (II) tetrafluoroborate hexahydrate, cobalt (II) tetrafluoroborate hexahydrate, cobalt (II) tetrafluoroborate hexahydrate, cobalt (II) thiocyanate, cobalt (II) thiocyanate hydrate, cobalt tris (2,2,6,6-tetramethyl-3,5-heptanedioate), cobalt (II) tungstate hydrate hydroxycobalamin (Vitamin B12).

41. Use of Claim 33 wherein said agent is manganese or nickel or pharmaceutically acceptable salts thereof or a compound containing manganese and/or nickel.

42. Use of Claim 41 wherein said manganese or manganese containing compound is selected from the list consisting of manganese, manganese(II) acetate, manganese(II) acetate tetrahydrate, manganese(III) acetate dihydrate, manganese(II) acetylacetone, manganese(III) acetylacetone, manganese(II) bromide, manganese(II) bromide
tetrahydrate, manganese carbonyl, manganese(II) chloride, manganese(II) chloride
tetrahydrate, manganese(II) cyclohexanbutyrate, manganese dioxide, manganese(II)
fluoride, manganese(III) fluoride, manganese(II) hexafluoroacetylacetonate trihydrate,
manganese(II) hydrogen phosphite, manganese(II) hypophosphate monohydrate,
manganese(II) iodide, manganese(II) nitrate hydrate, manganese(II) nitrate, manganese(II)
oxide, manganese(II, III) oxide, manganese (III) oxide, manganese(IV) oxide, manganese(IV)
oxide, 50% w/w on activated carbon, manganese(II) perchlorate
hexahydrate, manganese(II) phthalocyanine, manganese(III) phthalocyanine chloride,
manganese sesquioxide, manganese(II) sulfate hydrate, manganese(II) sulfate
monohydrate, manganese(II) sulfide, manganese(III) 5,10,15,20-tetra(4-pyridyl)-21 H ,23
H –porphine chloride tetrakis(methochloride), manganic acetylacetonate, manganocene
and manganous acetylacetonate.

43. Use of Claim 41 wherein said nickel or nickel containing compound is selected
from the list consisting of nickel, nickel(II) acetate tetrahydrate, nickel(II) acetylacetonate,
nickel(II) bis(2,2,6,6-tetramethyl-3,5-heptanedionate), nickel boride, nickel(II) bromide,
nickel(II) bromide hydrate, nickel(II) bromide ethylene glycol dimethyl ether complex,
nickel(II) bromide 2-methoxyethyl ether complex, nickel(II) carbonate, basic, nickel(II)
carbonate hydroxide tetrahydrate, nickel(II) chloride, nickel(II) chloride hexahydrate,
nickel(II) chloride hydrate, nickel(II) cyclohexanbutyrate, nickel(II) 2-ethylhexanoate,
nickel(II) fluoride, nickel(II) fluoride tetrahydrate, nickel(II) formate dihydrate, nickel(II)
hexafluoroacetylacetonate hydrate, nickel(II) hydroxide, nickel(II) iodide, nickel(II) nitrate
hexahydrate, nickel(II) 5,9,14,18,23,27,32,36-octabutoxy-2,3-naphthalocyanine, nickel(II)
1,4,8,11,15,18,22,25-octabutoxy-29H ,31 H –phthalocyanine, nickel(II) octanoate hydrate,
nickel, ~60% w/w on kieselghur, nickel, ~65% w/w on silica/alumina, nickel(II) oxalate
dihydrate, nickel(II) oxide, nickel(II) perchorate hexahydrate, nickel peroxide hydrate,
nickel phosphide, nickel(II) phthalocyanine, nickel(II) phthalocyaninotetrafulfonic acid,
tetrasodium salt, nickel(II) sulfamate tetrahydrate, nickel(II) sulfate heptahydrate,
nickel(II) sulfate hexahydrate, nickel sulfide, nickel(II) 2,11,20,29-tetra- tert –butyl-2,3-
naphthalocyanine, nickel(II) tetrafluoroborate, nickel(II) tetrakis(4-
cumylphenoxy)phthalocyanine, nickel(II) 2,9,16,23-tetraphenoxy-29H , 31 H –
phthalocyanine, nickel(II) titanate, nickel TPP and Ni(COD)$_2$.

44. A drug delivery device comprising a slowly releasable agent selected from the list consisting of a transition metal or compound which has the same or similar redox potential as, and can displace, iron in hemoglobin or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound.

45. The drug delivery device of Claim 44 wherein said agent is cobalt or a pharmaceutically acceptable salt thereof or a compound containing cobalt.

46. The drug delivery device of Claim 45 wherein said cobalt or cobalt-containing compound is selected from the list consisting of cobalt, cobalt (II) acetate, cobalt (II) acetate tetrahydrate, cobalt (II) acetylacetone, cobalt (ii) acetylacetone hydrate, cobalt (III) acetylacetone, cobalt (III) benzoyleacetone, cobalt boride, cobalt (II) bromide, cobalt (II) bromide hydrate, cobalt (II) carbonate hydrate, cobalt (II) chloride, cobalt (II) chloride hydrate, cobalt (II) chloride hexahydrate, cobalt (II) 2-ethylhexanoate, cobalt (II) fluoride, cobalt (II) fluoride tetrahydrate, cobalt (III) fluoride, cobalt (II) 1,2,3,4,8,9,10,11,15,16,17,18,22,23,24,25-hexadeca-fluoro-29H,31H-phthalocyanine, cobalt (II) hexafluoroacetylacetone hydrate, cobalt (II) hydroxide, cobalt (II) iodide, cobalt (II) 2,3-naphthalocyanine, cobalt (II) nitrate hexahydrate, cobalt (II) oxalate dihydrate, cobalt (II) oxide, cobalt (II,III) oxide, cobalt (II) perchlorate hexahydrate, cobalt (II) phthalocyanine, cobalt (II) selenide, cobalt (III) sephulchrate trichloride, cobalt (III) sephulchrate tris (tetraphenylborate), cobalt (II) sulfate hydrate, cobalt (II) tetrafluoroborate hexahydrate, cobalt (II) tetrafluoroborate hexahydrate, cobalt (II) thiocyanate, cobalt (II) thiocyanate hydrate, cobalt tris (2,2,6,6-tetramethyl-3,5-heptanedioate), cobalt (II) tungstate hydrate and hydroxycobalamin (Vitamin B12).

47. The drug delivery device of Claim 45 wherein said agent is manganese or nickel or
pharmaceutically acceptable salts thereof or a compound containing manganese and/or nickel.

48. The drug delivery device of Claim 47 wherein said manganese or manganese containing compound is selected from the list consisting of manganese, manganese(II) acetate, manganese(II) acetate tetrahydrate, manganese(III) acetate dihydrate, manganese(II) acetylacetonate, manganese(III) acetylacetonate, manganese(II) bromide, manganese(II) bromide tetrahydrate, manganese carbonyl, manganese(II) chloride, manganese(II) chloride tetrahydrate, manganese(II) cyclohexanbutyrate, manganese dioxide, manganese(II) fluoride, manganese(III) fluoride, manganese(II) hexafluoroacetylacetonate trihydrate, manganese(II) hydrogen phosphite, manganese(II) hypophosphite monohydrate, manganese(II) iodide, manganese(II) nitrate hydrate, manganese(II) nitrate, manganese(II) oxide, manganese(II, III) oxide, manganese (III) oxide, manganese(IV) oxide, manganese(IV) oxide, 50% w/w on activated carbon, manganese(II) perchlorate hexahydrate, manganese(II) phthalo cyanine, manganese(III) phthalocyanine chloride, manganese sesquioxide, manganese(II) sulfate hydrate, manganese(II) sulfate monohydrate, manganese(II) sulfide, manganese(III) 5,10,15,20-tetra(4-pyridyl)-21H, 23 H-porphine chloride tetrakis(methochloride), managanic acetylacetonate, mananganene and mananious acetylacetonate.

49. The drug delivery device of Claim 47 wherein said nickel or nickel containing compound is selected from the list consisting of nickel, nickel(II) acetate tetrahydrate, nickel(II) acetylacetonate, nickel(II) bis(2,2,6,6-tetramethyl-3,5-heptanedionate), nickel boride, nickel(II) bromide, nickel(II) bromide hydrate, nickel(II) bromide ethylene glycol dimethyl ether complex, nickel(II) bromide 2-methoxyethyl ether complex, nickel(II) carbonate, basic, nickel(II) carbonate hydroxide tetrahydrate, nickel(II) chloride, nickel(II) chloride hexahydrate, nickel(II) chloride hydrate, nickel(II) cyclohexanbutyrate, nickel(II) 2-ethylhexanoate, nickel(II) fluoride, nickel(II) fluoride tetrahydrate, nickel(II) formate dihydrate, nickel(II) hexafluoroacetylacetonate hydrate, nickel(II) hydroxide, nickel(II) iodide, nickel(II) nitrate hexahydrate, nickel(II) 5,9,14,18,23,27,32,36-octabutoxy-2,3-naphthalocyanine, nickel(II) 1,4,8,11,15,18,22,25-octabutoxy-29H,31 H-phthalocyanine,
nickel(II) octanoate hydrate, nickel, ~60% w/w on kieselghur, nickel, ~65% w/w on silica/alumina, nickel(II) oxalate dihydrate, nickel(II) oxide, nickel(II) perchlorate hexahydrate, nickel peroxide hydrate, nickel phosphide, nickel(II) phthalocyanine, nickel(II) phthalocyaninetetraculfsionic acid, tetrasodium salt, nickel(II) sulfamate tetrahydrate, nickel(II) sulfate heptahydrate, nickel(II) sulfate hexahydrate, nickel sulfide, nickel(II) 2,11,20,29-tetra- tert -butyl-2,3-naphthalocyanine, nickel(II) tetrafluoroborate, nickel(II) tetrakis(4-cumylphenoxy)phthalocyanine, nickel(II) 2,9,16,23-tetraphenoxy-29H, 31 H —phthalocyanine, nickel(II) titanate, nickel TPP and Ni(COD)₂.

50. The drug delivery device of Claim 44 wherein said device is a catheter.

51. The drug delivery device of Claim 44 wherein said device is a stent.

52. A method for promoting angiogenesis or vasculogenesis in a subject said method comprising administering to said subject an effective amount of an agent or combination of agents which elevate levels of stem cells or precursor cells thereof or early committed cells of a particular lineage, enhance angiogenic or vasculogenic capability of said cells and/or which mobilize said cells to a site in the systemic vasculature.

53. The method of Claim 52 wherein said stem cells are endothelial precursor cells (EPCs) or precursor cells thereof or early committed cells in an EPC lineage.

54. The method of Claim 53 wherein said stem cells are EPCs.

55. The method of Claim 52 wherein said subject is a mammal.

56. The method of Claim 55 wherein said mammal is a human.

57. The method of Claim 52 wherein said agent is selected from the list consisting of a transition metal or compound which has the same or similar redox potential as, and can displace, iron in hemoglobin or an iron atom-dependent dioxygenase by complexing with
the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound.

58. The method of Claim 52 wherein said agent stabilizes or otherwise elevates or maintains levels of a HIF protein.

59. The method of Claim 58 wherein said HIF protein is HIF-1.

60. The method of Claim 58 wherein said HIF protein is HIF-1α.

61. The method of Claim 52 wherein said agent is cobalt or a pharmaceutically acceptable salt thereof or a compound containing cobalt.

62. The method of Claim 61 wherein the cobalt or cobalt-containing compound is selected from the list consisting of cobalt, cobalt (II) acetate, cobalt (II) acetate tetrahydrate, cobalt (II) acetylacetonate, cobalt (ii) acetylacetonate hydrate, cobalt (III) acetylacetonate, cobalt (III) benzoylacetonate, cobalt boride, cobalt (II) bromide, cobalt (II) bromide hydrate, cobalt (II) carbonate hydrate, cobalt (II) chloride, cobalt (II) chloride hydrate, cobalt (II) chloride hexahydrate, cobalt (II) 2-ethylhexanoate, cobalt (II) fluoride, cobalt (II) fluoride tetrahydrate, cobalt (III) fluoride, cobalt (II) 1,2,3,4,8,9,10,11,15,16,17,18,22,23,24,25-hexadeca-fluoro-29H,31H-phthalocyanine, cobalt (II) hexafluoroacetylacetonate hydrate, cobalt (II) hydroxide, cobalt (II) iodide, cobalt (II) 2,3-naphthalocyanine, cobalt (II) nitrate hexahydrate, cobalt (II) oxalate dihydrate, cobalt (II) oxide, cobalt (II,III) oxide, cobalt (II) perchlorate hexahydrate, cobalt (II) phthalocyanine, cobalt (II) selenide, cobalt (III) sephulchraste trichloride, cobalt (III) sephulchraste tris (tetraphenylboraate), cobalt (II) sulfate hydrate, cobalt (II) tetrafluoroborate hexahydrate, cobalt (II) tetrafluoroborate hexahydrate, cobalt (II) tetrafluoroborate hexahydrate, cobalt (II) thiocyanate, cobalt (II) thiocyanate hydrate, cobalt tris (2,2,6,6-tetramethyl-3,5-heptanedionate), cobalt (II) tungstate hydrate and hydroxycobalamin (Vitamin B12).
63. The method of Claim 52 wherein said agent is manganese or nickel or pharmaceutically acceptable salts thereof or a compound containing manganese and/or nickel.

64. The method of Claim 63 wherein said manganese or manganese containing compound is selected from the list consisting of manganese, manganese(II) acetate, manganese(II) acetate tetrahydrate, manganese(III) acetate dihydrate, manganese(II) acetylacetone, manganese(III) acetylacetone, manganese(II) bromide, manganese(II) bromide tetrahydrate, manganese carbonyl, manganese(II) chloride, manganese(II) chloride tetrahydrate, manganese(II) cyclohexanbutyrate, manganese dioxide, manganese(II) fluoride, manganese(III) fluoride, manganese(II) hexafluoroacetylacetone trihydrate, manganese(II) hydrogen phosphite, manganese(II) hypophosphite monohydrate, manganese(II) iodide, manganese(II) nitrate hydrate, manganese(II) nitrate, manganese(II) oxide, manganese(II, III) oxide, manganese (III) oxide, manganese(IV) oxide, manganese(IV) oxide, 50% w/w on activated carbon, manganese(II) perchlorate hexahydrate, manganese(II) phthalocyanine, manganese(III) phthalocyanine chloride, manganese sesquioxide, manganese(II) sulfate hydrate, manganese(II) sulfate monohydrate, manganese(II) sulfide, manganese(III) 5,10,15,20-tetra(4-pyridyl)-21 H ,23 H –porphine chloride tetrakis(methochloride), manganic acetylacetonate, manganocene and manganous acetylacetonate.

65. The method of Claim 63 wherein said nickel or nickel containing compound is selected from the list consisting of nickel, nickel(II) acetate tetrahydrate, nickel(II) acetylacetone, nickel(II) bis(2,2,6,6-tetramethyl-3,5-heptanedionate), nickel boride, nickel(II) bromide, nickel(II) bromide hydrate, nickel(II) bromide ethylene glycol dimethyl ether complex, nickel(II) bromide 2-methoxyethyl ether complex, nickel(II) carbonate, basic, nickel(II) carbonate hydroxide tetrahydrate, nickel(II) chloride, nickel(II) chloride hexahydrate, nickel(II) chloride hydrate, nickel(II) cyclohexanebutyrate, nickel(II) 2-ethylhexanoate, nickel(II) fluoride, nickel(II) fluoride tetrahydrate, nickel(II) formate dihydrate, nickel(II) hexafluoroacetylacetone hydrate, nickel(II) hydroxide, nickel(II)
iodide, nickel(II) nitrate hexahydrate, nickel(II) 5,9,14,18,23,27,32,36-octabutoxy-2,3-naphthalocyanine, nickel(II) 1,4,8,11,15,18,22,25-octabutoxy-29H,31 H-phthalocyanine, nickel(II) octanoate hydrate, nickel, ~60% w/w on kieselguhr, nickel, ~65% w/w on silica/alumina, nickel(II) oxalate dihydrate, nickel(II) oxide, nickel(II) perchlorate hexahydrate, nickel peroxide hydrate, nickel phosphide, nickel(II) phthalocyanine, nickel(II) phthalocyaninetraculfonic acid, tetrasodium salt, nickel(II) sulfamate tetrahydrate, nickel(II) sulfate heptahydrate, nickel(II) sulfate hexahydrate, nickel sulfide, nickel(II) 2,11,20,29-tetra- tert-butyl-2,3-naphthalocyanine, nickel(II) tetrafluoroborate, nickel(II) tetrakis(4-cumylphenoxy)phthalocyanine, nickel(II) 2,9,16,23-tetraphenoxy-29H, 31 H-phthalocyanine, nickel(II) titanate, nickel TPP and Ni(COD)₂.

66. The method of Claim 52 wherein said agent is hydralazine or hydralazine-related compounds.

67. The method of Claim 52 wherein the effective amount of the agent is from about 10µM to about 2000µM.

68. The method of Claim 52 or 67 wherein the mode of administration is via topical, pulmonary, oral or parental administration.

69. The method of Claim 68 wherein parental administration is via intravenous, perivascular, intraarterial, intracoronary, intrapericardial, intraperitoneal, intracranial, intramyocardial, catheter or stent administration.

70. A method for the treatment or prophylaxis of infection by a pathogenic agent in a subject, said method comprising administering to said subject an effective amount of an agent selected from the list consisting of a transition metal or compound which has the same or similar redox potential as, and can displace, iron in hemoglobin or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition
metal or compound; hydralazine; and a hydralazine-related compound.

71. The method of Claim 70 wherein said agent stabilizes or otherwise elevates or maintains levels of a HIF protein.

72. The method of Claim 71 wherein said HIF protein is HIF-1.

73. The method of Claim 72 wherein said HIF protein is HIF-1α.

74. The method of 70 wherein said agent is cobalt or a pharmaceutically acceptable salt thereof or a compound containing cobalt.

75. The method of Claim 74 wherein said cobalt or cobalt-containing compound is selected from the list consisting of cobalt, cobalt (II) acetate, cobalt (II) acetate tetrahydrate, cobalt (II) acetylacetonate, cobalt (II) acetylacetonate hydrate, cobalt (III) acetylacetonate, cobalt (III) benzoylacetonate, cobalt boride, cobalt (II) bromide, cobalt (II) bromide hydrate, cobalt (II) carbonate hydrate, cobalt (II) chloride, cobalt (II) chloride hydrate, cobalt (II) chloride hexahydrate, cobalt (II) 2-ethylhexanoate, cobalt (II) fluoride, cobalt (II) fluoride tetrahydrate, cobalt (III) fluoride, cobalt (II) 1,2,3,4,8,9,10,11,15,16,17,18,22,23,24,25-hexadeca-fluoro-29H,31H-phthalocyanine, cobalt (II) hexafluoroacetacetonate hydrate, cobalt (II) hydroxide, cobalt (II) iodide, cobalt (II) 2,3-napthalocyanine, cobalt (II) nitrate hexahydrate, cobalt (II) oxalate dihydrate, cobalt (II) oxide, cobalt (II,III) oxide, cobalt (II) perchlorate hexahydrate, cobalt (II) phthalocyanine, cobalt (II) selenide, cobalt (III) sephulchrate trichloride, cobalt (III) sephulchrate tris (tetrphenyborate), cobalt (II) sulfate hydrate, cobalt (II) tetrafluoroborate hexahydrate, cobalt (II) tetrathiocyanatomercurate, cobalt (II) thiocyanate, cobalt (II) thiocyanate hydrate, cobalt tris (2,2,6,6-tetramethyl-3,5-heptanedioate), cobalt (II) tungstate hydrate and hydroxycobalamin (Vitamin B12).

76. The method of Claim 70 wherein said agent is manganese or nickel or pharmaceutically acceptable salts thereof or a compound containing manganese and/or
nickel.

77. The method of Claim 76 wherein said manganese or manganese containing compound is selected from the list consisting of manganese, manganese(II) acetate, manganese(II) acetate tetrahydrate, manganese(III) acetate dihydrate, manganese(II) acetylacetonate, manganese(III) acetylacetonate, manganese(II) bromide, manganese(II) bromide tetrahydrate, manganese carbonyl, manganese(II) chloride, manganese(II) chloride tetrahydrate, manganese(II) cyclohexanobutyrate, manganese dioxide, manganese(II) fluoride, manganese(III) fluoride, manganese(II) hexafluoroacetylacetonate trihydrate, manganese(II) hydrogen phosphite, manganese(II) hypophosphite monohydrate, manganese(II) iodide, manganese(II) nitrate hydrate, manganese(II) nitrate, manganese(II) oxide, manganese(II, III) oxide, manganese (III) oxide, manganese(IV) oxide, manganese(IV) oxide, 50% w/w on activated carbon, manganese(II) perchlorate hexahydrate, manganese(II) phthalocyanine, manganese(III) phthalocyanine chloride, manganese sesquioxide, manganese(II) sulfate hydrate, manganese(II) sulfate monohydrate, manganese(II) sulfide, manganese(III) 5,10,15,20-tetra(4-pyridyl)-21 $H$,23 $H$-porphine chloride tetraakis(methochloride), manganic acetylacetonate, manganocene and manganous acetylacetonate.

78. The method of Claim 76 wherein said nickel or nickel containing compound is selected from the list consisting of nickel, nickel(II) acetate tetrahydrate, nickel(II) acetylacetonate, nickel(II) bis(2,2,6,6-tetramethyl-3,5-heptanedionate), nickel boride, nickel(II) bromide, nickel(II) bromide hydrate, nickel(II) bromide ethylene glycol dimethyl ether complex, nickel(II) bromide 2-methoxyethyl ether complex, nickel(II) bromide hydrate, nickel(II) chloride, nickel(II) chloride hexahydrate, nickel(II) chloride hydrate, nickel(II) cyclohexanobutyrate, nickel(II) 2-ethylhexanoate, nickel(II) fluoride, nickel(II) fluoride tetrahydrate, nickel(II) formate dihydrate, nickel(II) hexafluoroacetylacetonate hydrate, nickel(II) hydroxide, nickel(II) iodide, nickel(II) iodide hydrate, nickel(II) 5,9,14,18,23,27,32,36-octabutoxy-2,3-naphthalocyanine, nickel(II) 1,4,8,11,15,18,22,25-octabutoxy-29$H$,31$H$-phthalocyanine, nickel(II) octanoate hydrate, nickel, ~60% w/w on kieselghur, nickel, ~65% w/w on

79. The method of Claim 70 wherein said agent is hydralazine or hydralazine-related compounds.

80. The method of any one of Claims 70 to 79 wherein the effective amount of the agent is from about 10μM to about 2000μM.

81. The method of Claim 70 wherein the subject is a mammal.

82. The method of Claim 81 wherein the mammal is a human.

83. The method of Claim 70 or 82 wherein the pathogenic agent is a virus, prokaryote or a eukaryote.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.
A61K 33/00 (2006.01) A61K 33/32 (2006.01) A61P 9/00 (2006.01) A61P 31/00 (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, MEDLINE, PUBMED: cobalt, manganese, nickel, hydralazine, stem cells, endothelial cells, precursor cells, progenitor cells, EPC, vascular, vessels, circulation, angiogenesis, vasculogenesis, hypoxia-inducible factor or HIF and related terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>See whole document</td>
<td></td>
</tr>
<tr>
<td></td>
<td>See whole document</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>US 5480975 A (Goldberg et al) 2 January 1996</td>
<td>1-19, 33-43, 52-69</td>
</tr>
<tr>
<td></td>
<td>See whole document especially abstract; claims; col 1-3</td>
<td></td>
</tr>
</tbody>
</table>

[X] Further documents are listed in the continuation of Box C  [X] 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 claim 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
5 September 2007

Name and mailing address of the ISA/AU
AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2605, AUSTRALIA
E-mail address: pct@ipaustralia.gov.au
Facsimile No. (02) 6283 3929

Date of mailing of the international search report
11 SEP 2007

Authorized officer
KRYSTYNA WARYLO
AUSTRALIAN PATENT OFFICE
ISO 9001 Quality Certified Service
Telephone No : (02) 6283 2814

Form PCT/ISA/210 (second sheet) (April 2007)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>See whole document</td>
<td></td>
</tr>
<tr>
<td></td>
<td>See whole document</td>
<td></td>
</tr>
</tbody>
</table>
INTERNATIONAL SEARCH REPORT

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.: 1, 7, 33, 38, 52, 57  
   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:

   See Supplemental Box I

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:

   See Supplemental Box II

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.: 1-19, 33-43, 52-69

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.
Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: II

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty. So many documents were retrieved that it was impossible to determine which part of the claims may be said to define subject matter for which protection might legitimately be sought. For this reasons, a meaningful search over the whole breadth of the claims is impossible.

Presently, claims 1, 33 and 52 relate to a method and use an agent or combination of agents involving a large number of compounds and their seemingly endless combinations making it virtually impossible to determine the full scope and complete meaning of the claimed subject matter. As presented, the claims subject cannot be regarded as being a clear and concise description for which protection is sought. In particular, the scope for claims 1, 33 and 52 encompasses any candidate agent. This travels beyond the scope of the invention and therefore these claims also lack descriptive support. Thus it is impossible to carry out a meaningful search on all the claims. Consequently, the search has been restricted to the compounds mentioned in the examples and encompassing claims.

Furthermore, the present claims relate to methods and uses of for the treatment of an extremely large number of possible disorders and/or diseases by the use of the terms "...an event, condition and/or disease of, or affecting the systemic vasculature" due thereto, a lack of clarity (and/or conciseness) arises to an extent as to render a meaningful search over the complete scope of the claims impossible.

Similarly, it is impossible to draft a rational or economic search based on indefinite terms found in the claims such a "early committed cells of a particular lineage" of claims 1, 33 and 52 or "similar redox potential as—iron" and "a hydralazine-related compound" of claims 7, 38 and 57. Therefore the scope of the claims cannot be determined.

Consequently, the search has been carried out for those parts of the claims which appear to be clear (and/or concise), supported and disclosed, namely those parts relating to the agents specifically disclosed in the examples and claims.
Supplemental Box
(To be used where the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: III

The international application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept. In coming to this conclusion, the International Searching Authority has found that there are four inventions:

1. Claims 1-19, 33-43, 52-69 are directed to a use and/or method of treatment for conditions or disease of affecting the systemic vasculature with an agent or combination of agents which elevates the level of stem/precursor cells which enhances angiogenesis or vasculogenesis.

2. Claims 20-32 are directed to a method of stabilising a HIF protein in a cell by administering an agent selected from a transition metal or compound which has the same or similar redox potential as, and can displace, iron in haemoglobin or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound.

3. Claims 44-51 are directed to a drug delivery device comprising a slowly releasable agent selected from a transition metal or compound which has the same or similar redox potential as, and can displace, iron in haemoglobin or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound.

4. Claims 70-83 are directed to a method of treating an infection by a pathogenic agent in a subject comprising administering an agent selected from a transition metal or compound which has the same or similar redox potential as, and can displace, iron in haemoglobin or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound.

Since the abovementioned groups of claims do not share either of the technical features identified, a "technical relationship" between the inventions, as defined in PCT rule 13.2 does not exist. Accordingly the international application does not relate to one invention or to a single inventive concept.

In addition, if the agent in claims 1-19, 33-43, 52-69 is limited to an agent selected from a transition metal or compound which has the same or similar redox potential as, and can displace, iron in haemoglobin or an iron atom-dependent dioxygenase by complexing with the organic protoporphyrin ring and/or organic subunit of an iron-dependent dioxygenase and/or complexing with the inorganic subunit of an iron atom-dependent dioxygenase, a compound or complex containing said transition metal or compound; hydralazine; and a hydralazine-related compound, the common concept linking together these groups of claims is the agent. However this concept is not novel in the light of the following documents:


Consequently the common features do not constitute "a special technical feature" within the meaning of PCT Rule 13.2, second sentence, since they make no contribution over the prior art. Since there exists no other common feature which can be considered as a special technical feature within the meaning of PCT Rule 13.2, second sentence, no technical relationship within the meaning of PCT Rule 13 between the different inventions can be seen. Therefore, a posteriori, the claims do not satisfy the requirement of unity of invention.

As the search for the other three inventions will require more than a negligible additional search effort over that for the first invention, an additional search fee was warranted but not paid.
This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5480975</td>
<td></td>
</tr>
</tbody>
</table>

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX