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(54) **METHODS FOR TREATING HEMATOPOIETIC NEOPLASMS**

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(57) **ABSTRACT**

This invention relates to methods for treating, preventing and/or managing hematopoietic neoplasm in a subject by administering to the subject combretastatin A-4 phosphate, or a pharmaceutically acceptable salt thereof. The method may further comprise co-administering a chemotherapeutic agent.

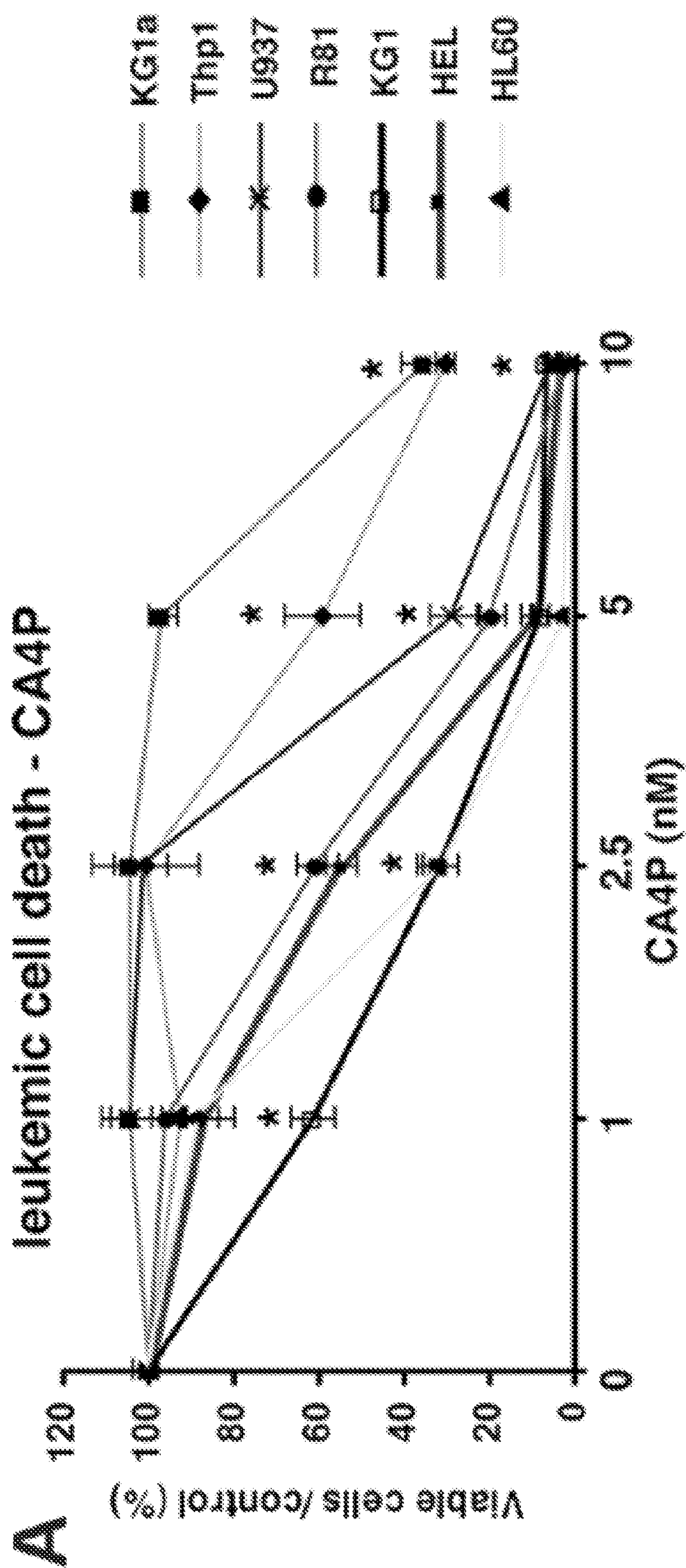


Figure 1

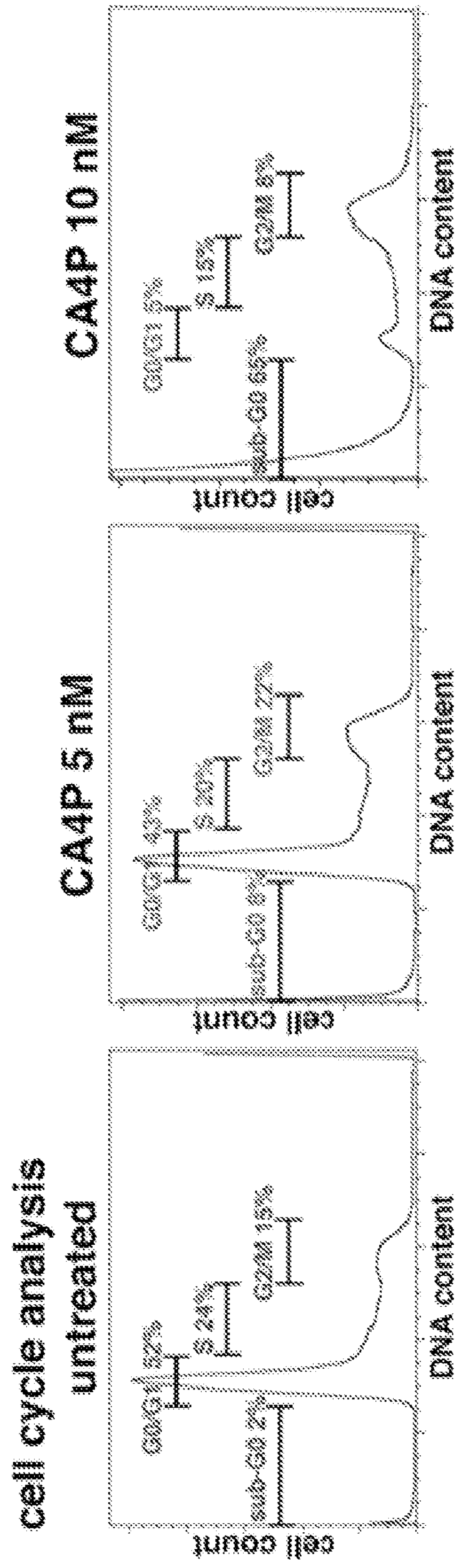


Figure 2

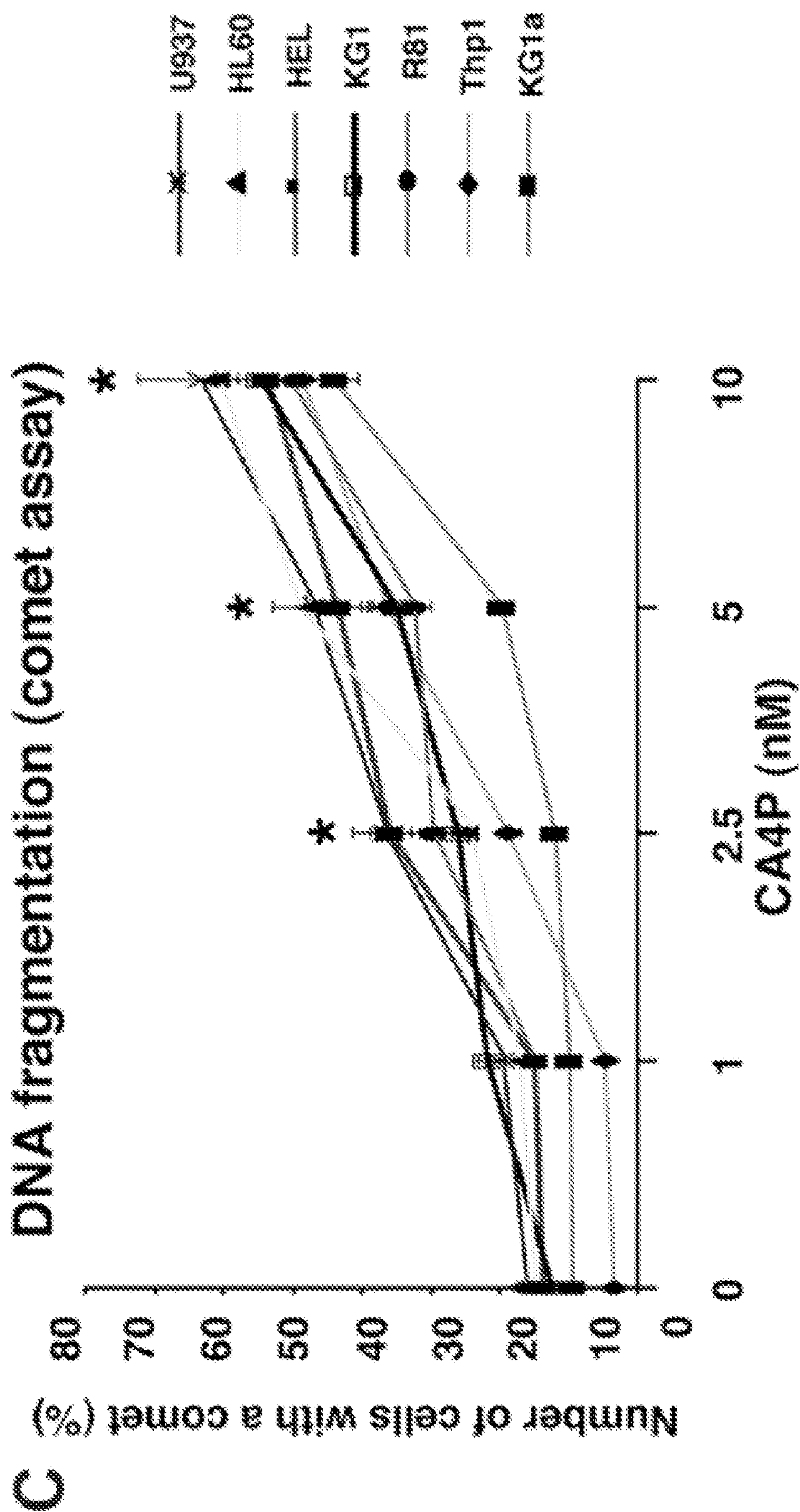


Figure 3

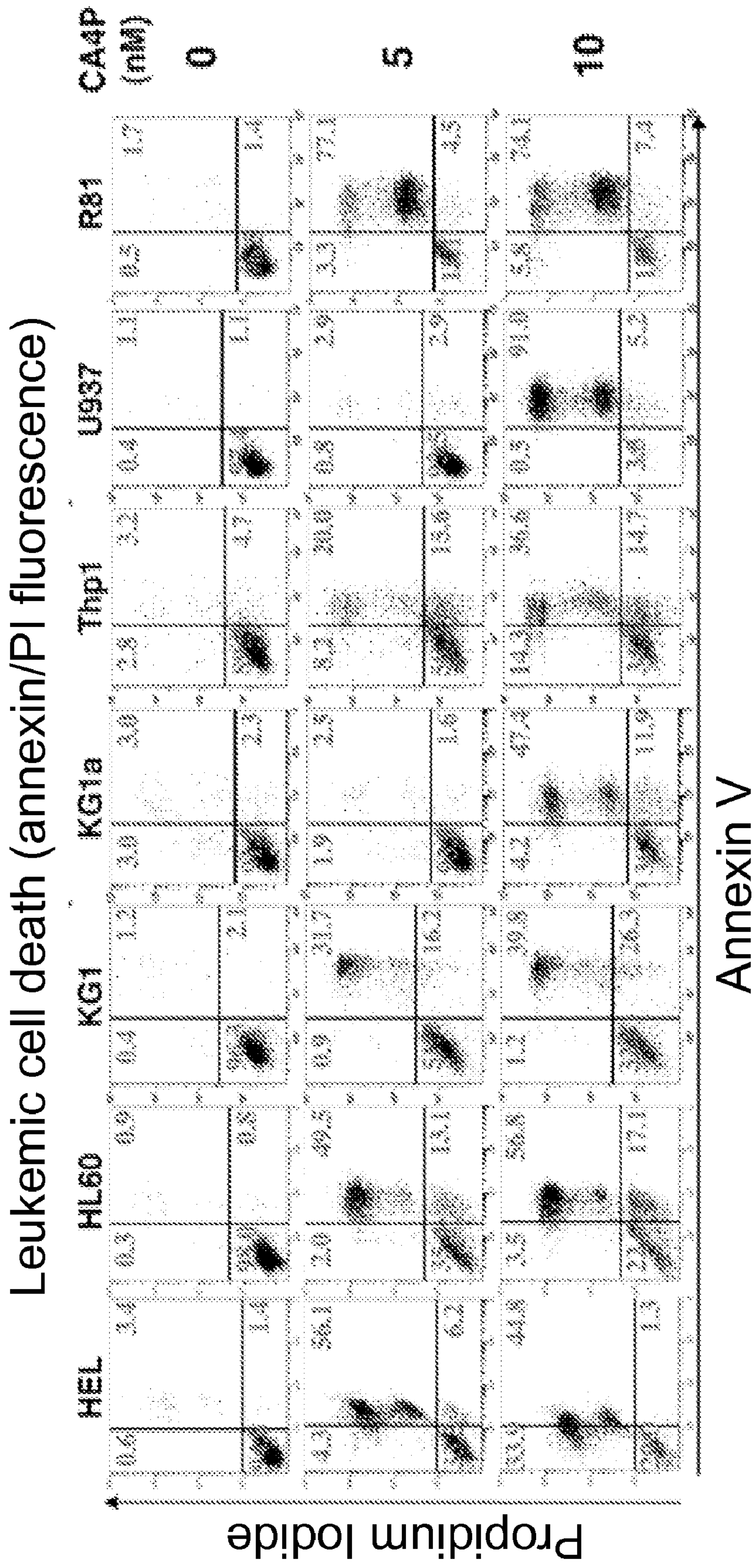


Figure 4

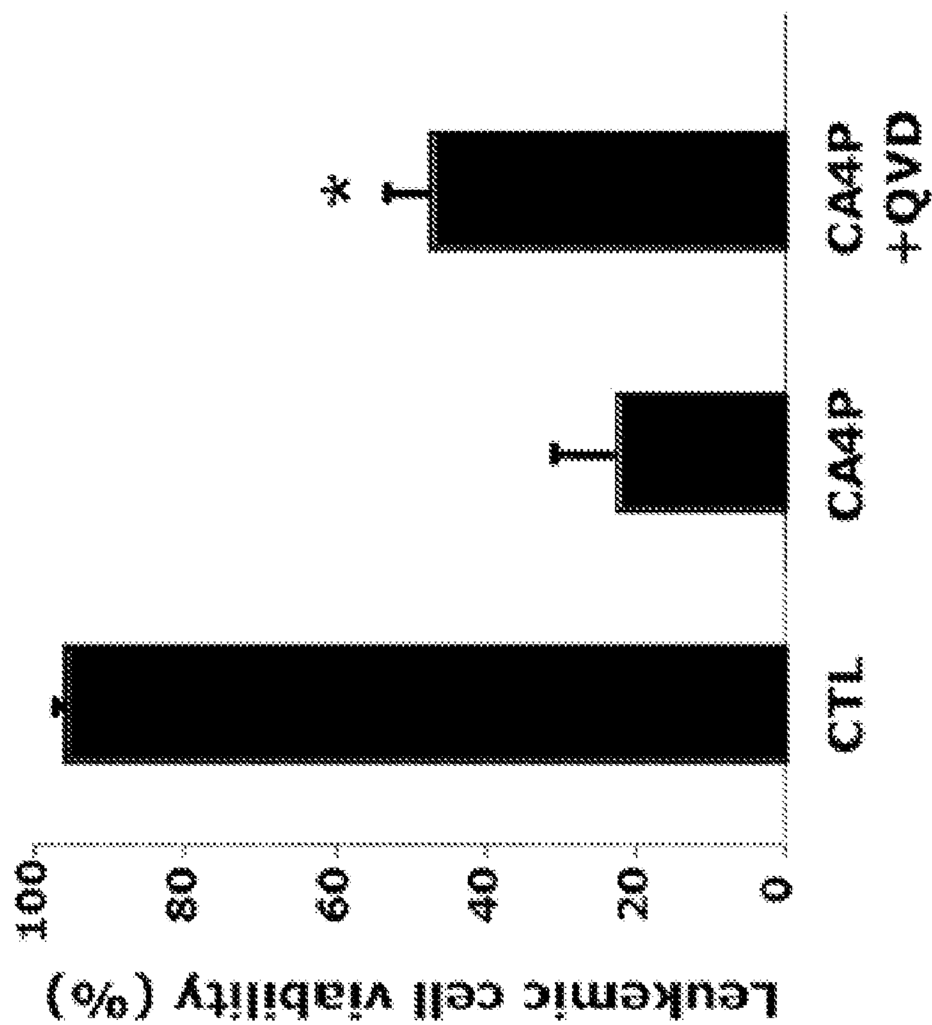


Figure 5

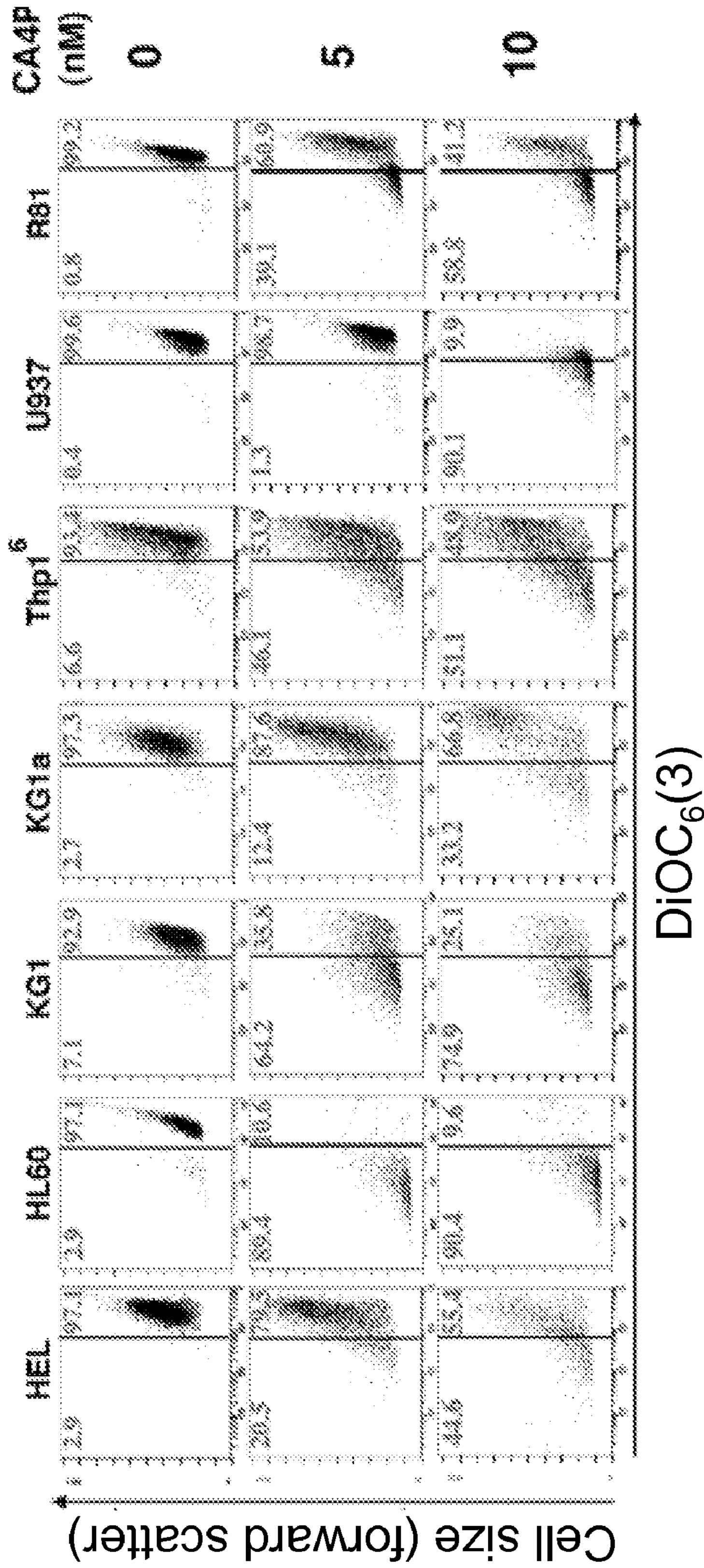


Figure 6

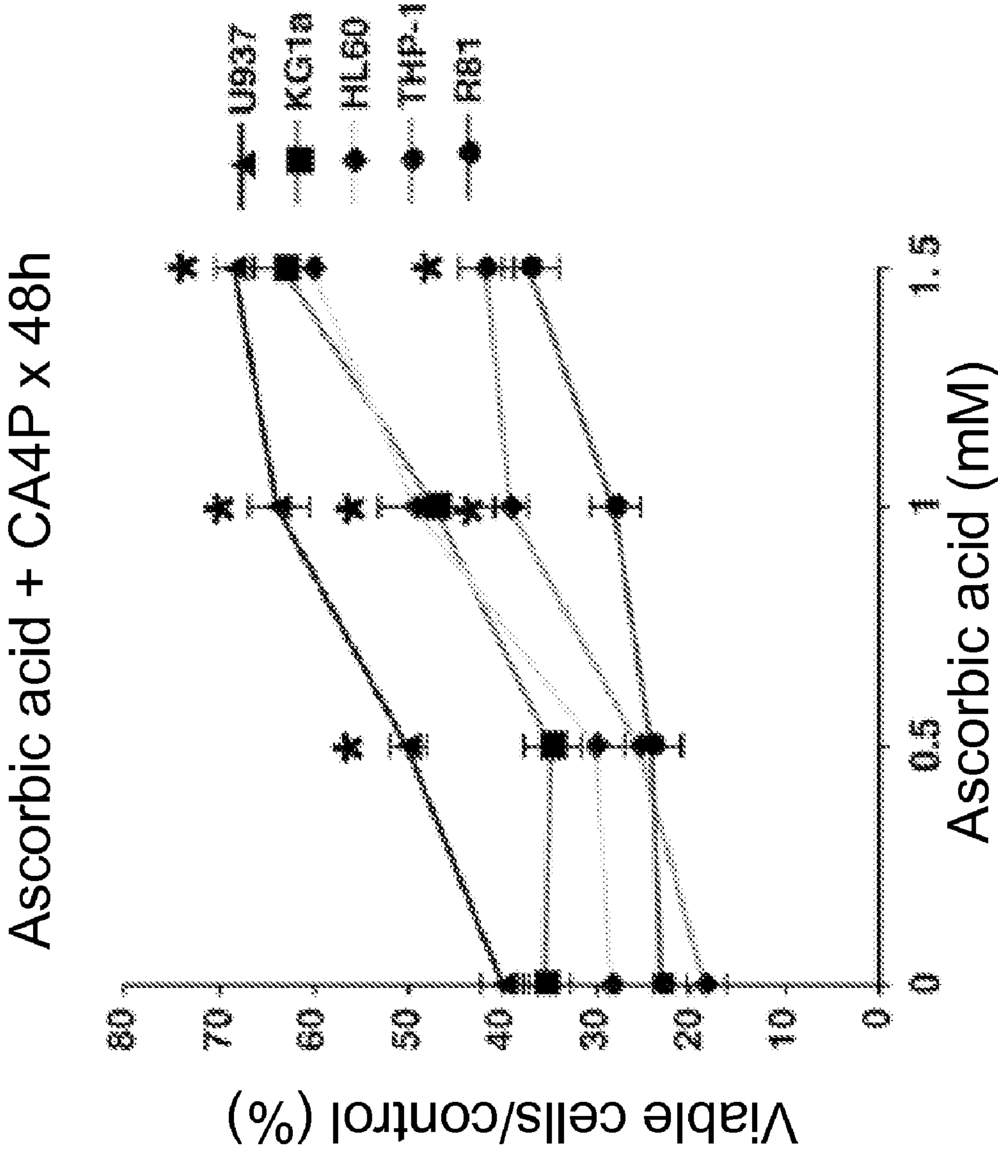


Figure 7

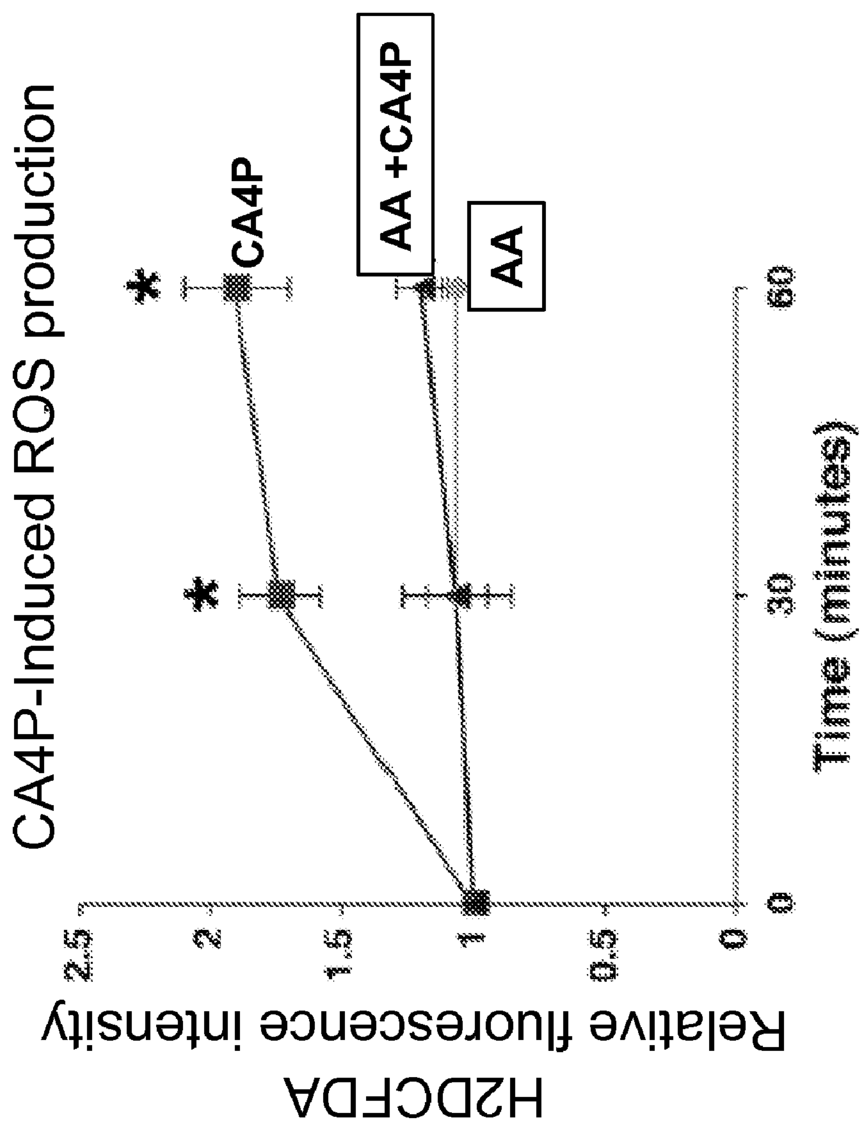


Figure 8

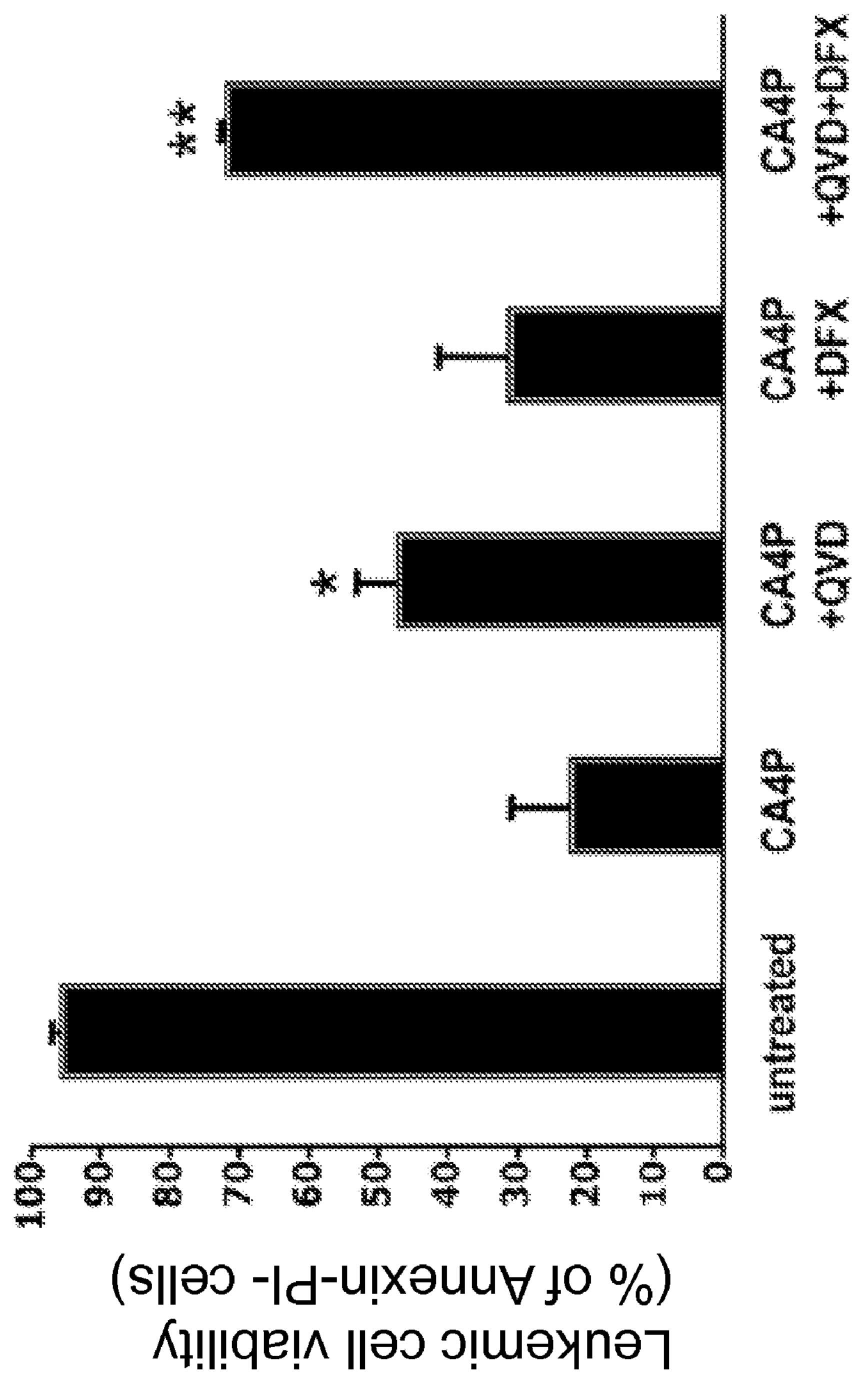


Figure 9

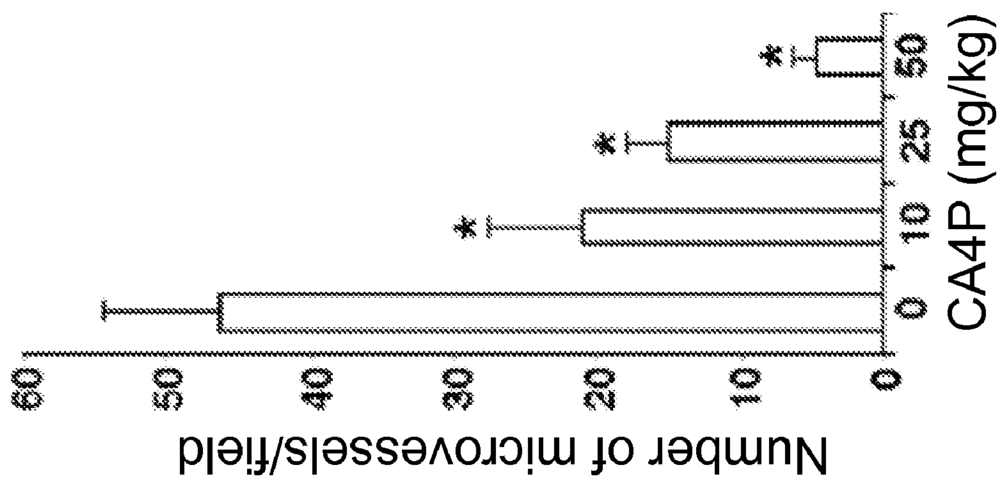


Figure 10

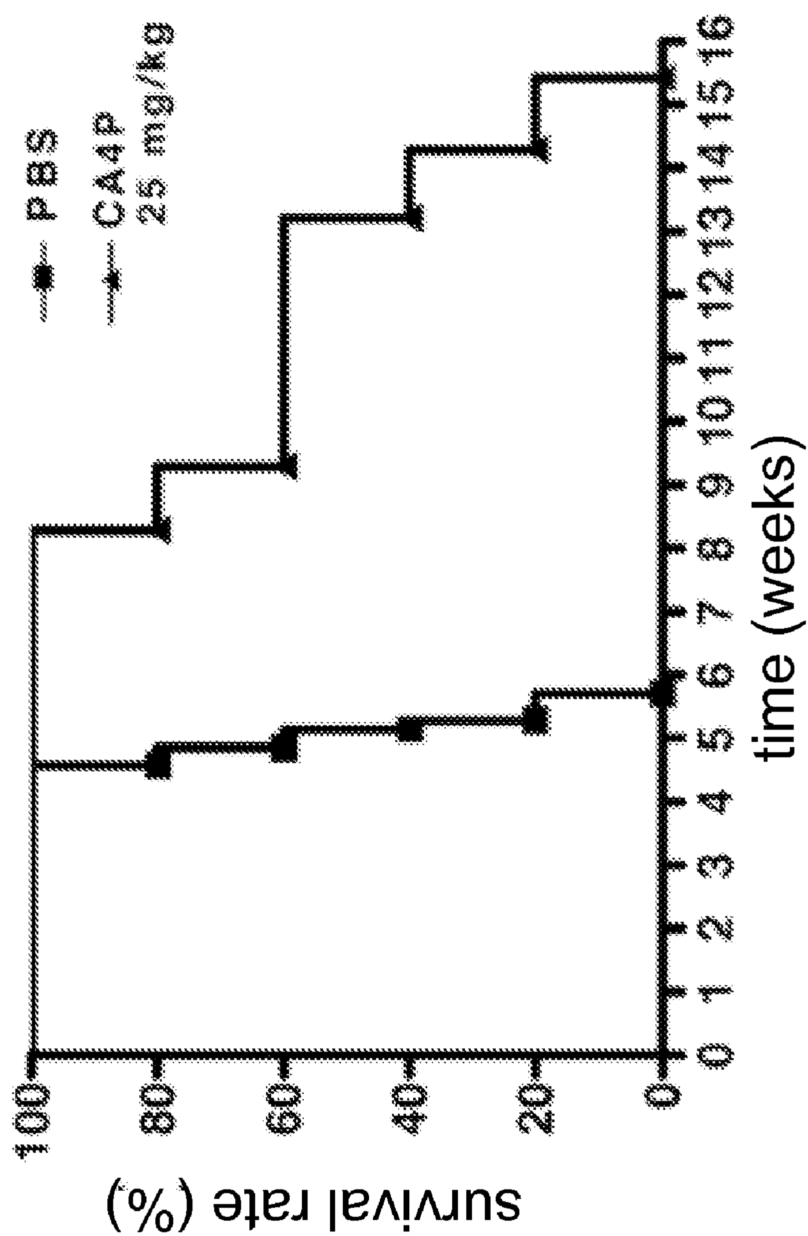


Figure 11

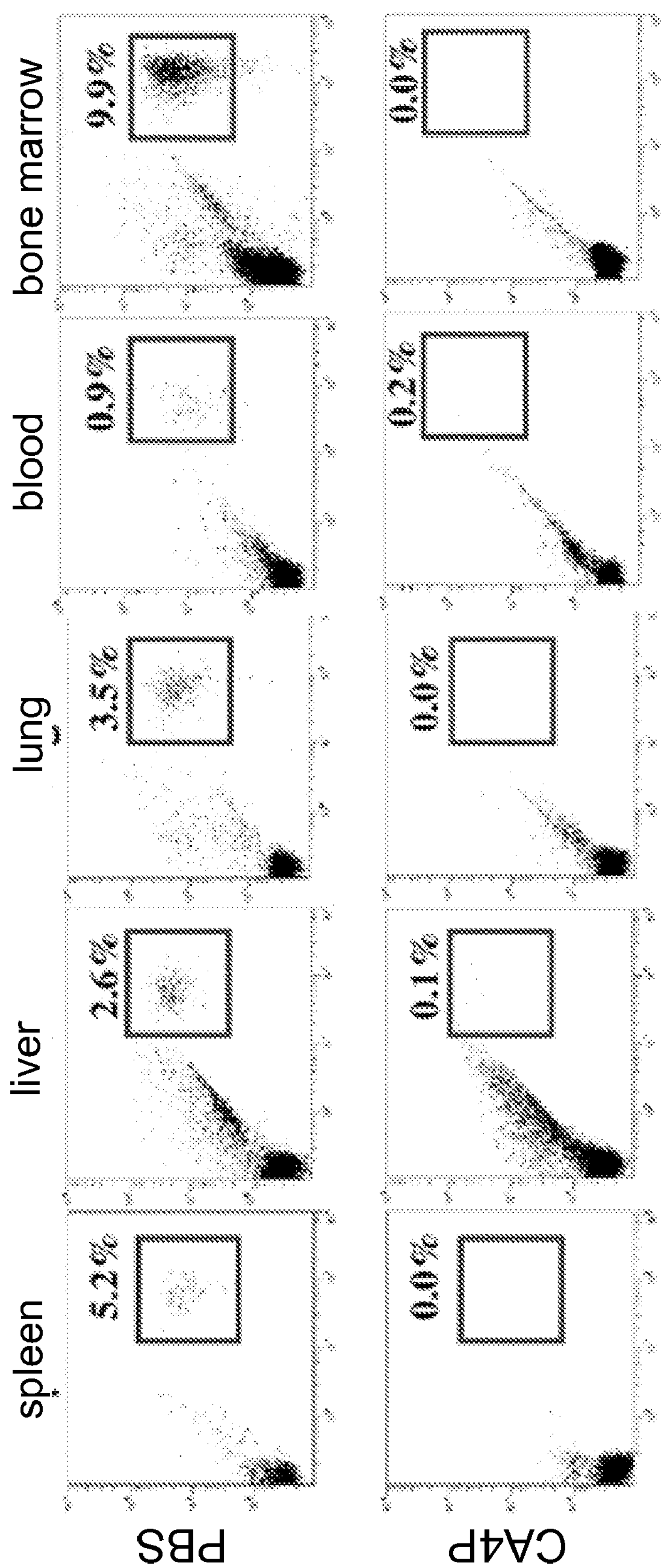


Figure 12

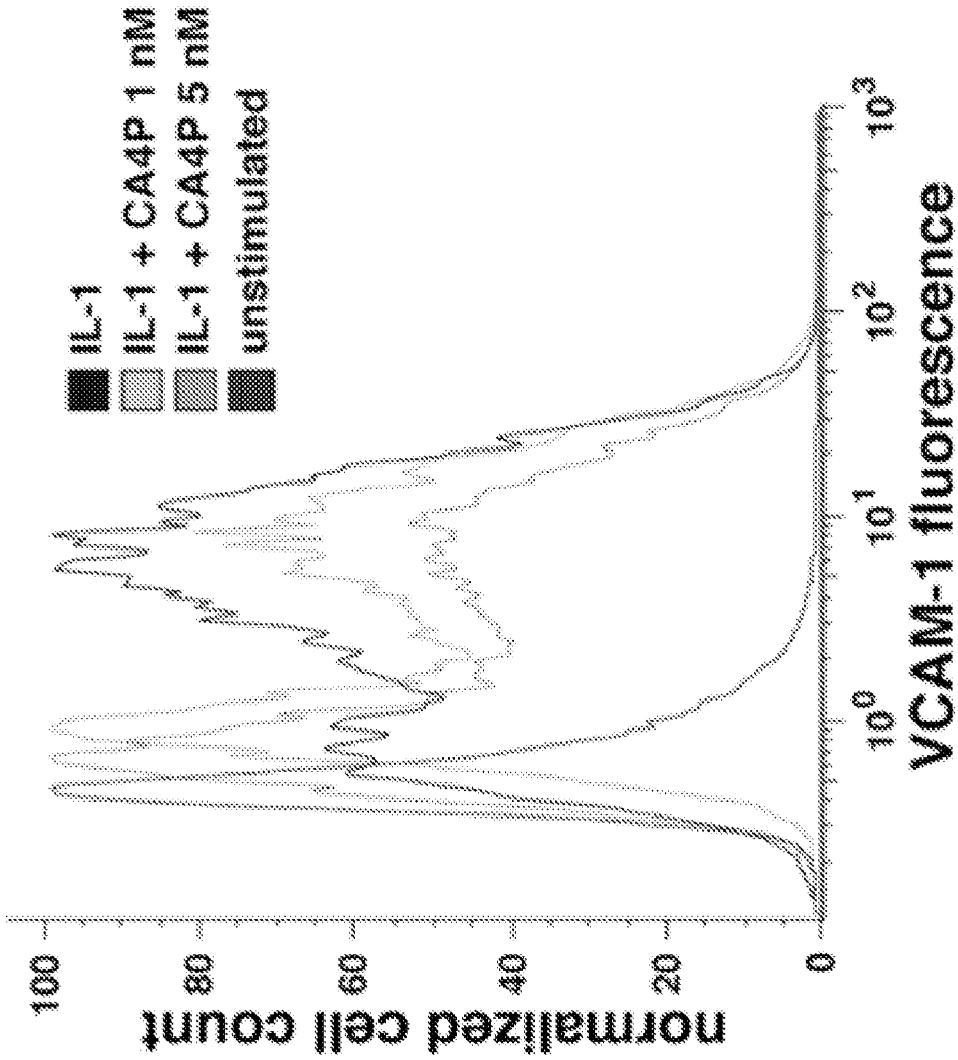


Figure 13

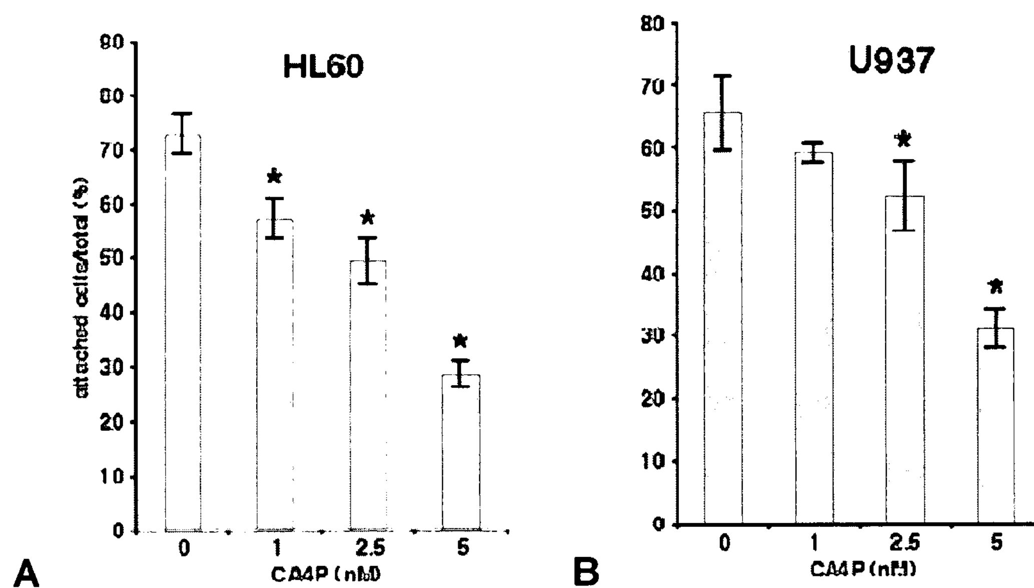


FIG. 14

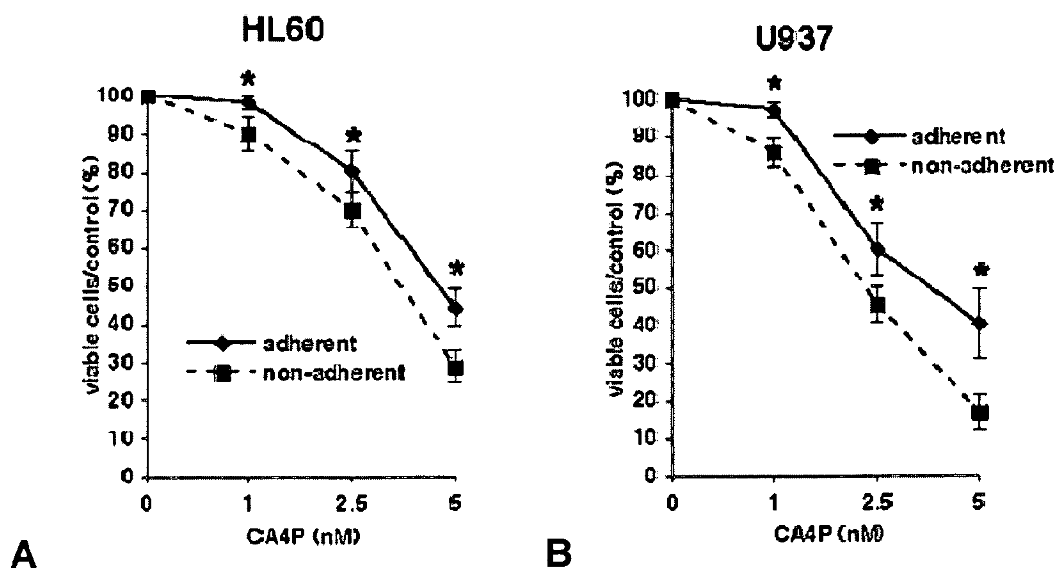


FIG. 15

METHODS FOR TREATING HEMATOPOIETIC NEOPLASMS

I. CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional patent application No. 60/989,786, filed 21 Nov. 2007.

II. INTRODUCTION

[0002] Although chemotherapy induces remission in the majority of adult patients with acute myeloid leukemia (AML), only small percent are cured with conventional chemotherapy. Relapse of leukemias is in part due to the persistence of minimal residual leukemias that remain viable within specialized niches, such as vascular niches. Hence, novel treatment strategies are urgently needed to block the interaction of hematopoietic neoplasms with activated vasculature, interfering with the establishment of pro-leukemic niches in various organs and to eradicate resistant disease.

[0003] Adhesion of leukemic cells to stromal cells has been shown to confer increased resistance to chemotherapeutic agents and diminish the rate of apoptosis of the leukemic cells. This process, named cell adhesion-mediated drug resistance (CAM-DR), depends on the interaction of integrins with their ligands. Adhesion of VLA4 (very late antigen 4, $\alpha 4\beta 1$) integrin-positive myeloid cells, to VCAM-1+ stromal cells is an important mediator of CAM-DR. Indeed, expression of VLA4 by leukemic cells portends a poor prognosis and a decreased five-year survival rate. Therefore, identification of novel anti-leukemic agents that inhibit interaction of leukemic cells with vascular cells provides novel strategies to target organ-infiltrating, angiogenesis-dependent leukemias.

[0004] Within the marrow or in circulation, hematopoietic neoplasms are closely associated with endothelium, supporting establishment of neo-vessels by elaboration of angiogenic factors. In addition, leukemic cells may activate endothelial cells by releasing pro-inflammatory factors, including interleukin-1 (IL-1), facilitating invasion into tissues and formation of infiltrative organ disease or subcutaneous tumors, namely chloromas, thereby establishing chemotherapy-refractory leukemic minimal residual disease.

[0005] One approach to destabilize interactions of hematopoietic neoplasms with endothelium is through disruption of the cytoskeletal organization of the neoplastic cells. Indeed, disruption of cytoskeletal stability of hematopoietic neoplasms may not only promote cell death directly, but also diminish the cellular interaction of the hematopoietic neoplasms with vascular cells, thereby increasing sensitivity to chemotherapy.

[0006] Combretastatin-A4, a novel tubulin-destabilizing agent, was isolated from the South African tree *Combreturn caffrum*. Combretastatin-A4 binds to tubulin at the same site as colchicine does, but with even higher affinity. Its pro-drug, combretastatin-A4 phosphate (CA4P) induces rapid microtubule depolymerization and vascular shutdown in subcutaneous solid tumors causing tumor necrosis at concentrations well below the maximum tolerated dose. CA4P also can induce apoptosis of the endothelial cells by disengaging VE-cadherin interaction. Thus, CA4P may not only target rapidly proliferating leukemic cells directly, but also diminish interaction of the leukemic cells with activated endothelial cells, thereby preventing establishment of a perivascular nidus for leukemic chloromas.

[0007] The inventors demonstrate that CA4P at low, non-toxic doses, surprisingly induces rapid cell death of non-adherent leukemic cells through caspase activation, mitochondria destabilization and accumulation of reactive oxygen species (ROS), accompanied by the release of pro-apoptotic mitochondrial membrane proteins. Additionally single-agent CA4P treatment is effective in eradicating both circulating, and vascular-adherent leukemic cells in subcutaneous and systemic mouse models of AML, without affecting normal hematopoiesis. CA4P-treated mice had significantly prolonged survival and showed a drastic reduction of detectable leukemic cells in the marrow and peripheral circulation, and significantly decreased leukemic organ infiltration. In addition, CA4P decreases expression of VCAM-1 on endothelial cells both in vitro and in vivo, thereby decreasing leukemic cell adhesion to the vascular cells, thereby reversing drug resistance. Therefore, CA4P delivered in combination with chemotherapeutic agents represents a promising novel therapeutic approach to treat hematopoietic neoplasms.

III. SUMMARY OF THE INVENTION

[0008] One aspect of the invention provides methods of treating a hematopoietic neoplasm comprising administering a therapeutically effective amount of combretastatin A-4 phosphate (CA4P), or a pharmaceutically acceptable salt thereof, to a subject having a hematological malignancy.

[0009] Another aspect of the invention provides the use of combretastatin A-4 phosphate, or a pharmaceutically acceptable salt thereof, for the treatment of a hematopoietic neoplasm. The invention also contemplates use of combretastatin A-4 phosphate and salts thereof in the preparation of a medicament for use in treating a hematopoietic neoplasm.

[0010] Yet another aspect of the invention provides methods of treating a non-solid tumor comprising administering a therapeutically effective amount of combretastatin A-4 phosphate or a pharmaceutically acceptable salt thereof, to a subject suffering from non-solid tumor.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. **1** illustrates combretastatin A-4 phosphate (CA4P) blocking leukemic cell growth of a panel of leukemic cell lines.

[0012] FIG. **2** illustrates CA4P induction of G2/M arrest.

[0013] FIG. **3** illustrate CA4P induction of DNA fragmentation in leukemic cells.

[0014] FIG. **4** demonstrates CA4P inducing cell death without evidence of necrosis.

[0015] FIG. **5** demonstrates that CA4P mediated apoptosis of leukemic cells is partially reversed by the caspase inhibitor Q-VD.

[0016] FIG. **6** illustrates the decrease in mitochondrial transmembrane potential of leukemic cells in response to exposure to CA4P.

[0017] FIG. **7** demonstrates that Combretastatin A-4 phosphate (CA4P) induced cell death can be partially prevented by co-incubation with ascorbic acid, an ROS scavenger, in a concentration-dependent manner.

[0018] FIG. **8** demonstrates early ROS accumulation during CA4P treatment.

[0019] FIG. **9** illustrates that CA4P mediated apoptosis of leukemic cells is reversed by inhibiting ROS and caspase pathways.

[0020] FIG. **10** provides quantification of the microvessel density in HL60 tumor sections after CA4P treatment.

[0021] FIG. **11** demonstrates that combretastatin A-4 phosphate (CA4P) improves survival of xenotransplanted mice with human leukemia cells.

[0022] FIG. **12** illustrates the CA4P-mediated decrease in leukemic cell circulation in the peripheral blood and engraftment in the bone marrow, spleen, liver and lung.

[0023] FIG. **13** demonstrates that CA4P inhibits IL-1-mediated upregulation of VCAM-1 in HUVECs.

[0024] FIGS. **14**A and **14**B demonstrate that CA4P reduces leukemic cell adhesion to HUVECs.

[0025] FIGS. **15**A and **15**B illustrate that leukemic cells adherent to HUVECs are more resistant to CA4P.

V. DETAILED DESCRIPTION

A. Definitions

[0026] As used herein, a “therapeutically effective amount” of combretastatin A-4 phosphate (CA4P), or a therapeutically acceptable salt thereof, according to the present invention is intended to mean that amount of the CA4P that will inhibit the growth of, or retard cancer, or kill malignant cells, and cause the regression and palliation of cancer, i.e., reduce the proliferation rate and/or the number of malignant cells within the body. Other desired anti-tumor effects include, without limitation, the modulation of neoplasm growth rates, the enhancement of necrosis or hypoxia in malignant cells, reduced retention of CEPs and other pro-angiogenic cells, amelioration or minimization of the clinical impairment or symptoms of hematopoietic neoplasms, extending the survival of the subject beyond that which would otherwise be expected in the absence of such treatment, and the prevention of neoplastic growth in an animal lacking any neoplasm formation prior to administration, i.e., prophylactic administration.

[0027] As used herein, the terms “modulate”, “modulating” or “modulation” refer to changing the rate at which a particular process occurs, inhibiting a particular process, reversing a particular process, and/or preventing the initiation of a particular process. Accordingly, if the particular process is neoplastic growth or metastasis, the term “modulation” includes, without limitation, decreasing the rate at which neoplastic growth and/or metastasis occurs; inhibiting neoplastic growth and/or metastasis, including tumor re-growth following treatment with an anticancer agent; reversing neoplastic growth and/or metastasis (including tumor shrinkage and/or eradication) and/or preventing neoplastic growth and/or metastasis.

[0028] “Hematopoietic neoplasm” refers to a cell proliferative disorder arising from cells of the hematopoietic lineage. Generally, hematopoiesis is the physiological process whereby undifferentiated cells or stem cells develop into various cells found in the peripheral blood. In the initial phase of development, hematopoietic stem cells, typically found in the bone marrow, undergo a series of cell divisions to form multipotent progenitor cells that commit to two main developmental pathways: the lymphoid lineage and the myeloid lineage. The committed progenitor cells of the myeloid lineage differentiate into three major sub-branches comprised of the erythroid, megakaryocyte, and granulocyte/monocyte developmental pathways. An additional pathway leads to formation of dendritic cells, which are involved in antigen presentation. The erythroid lineage gives rise to red blood cells while the megakaryocytic lineage gives rise to blood platelets. Committed cells of the granulocyte/monocyte lineage split into granulocyte or monocyte developmental pathways, the former pathway leading to formation of neutrophils, eosinophils, and basophils and the latter pathway giving rise to blood monocytes and macrophages.

[0029] Neoplasms of hematopoietic cells can involve cells of any phase of hematopoiesis, including hematopoietic stem cells, multipotent progenitor cells, oligopotent committed progenitor cells, precursor cells, and mature differentiated cells. The categories of hematopoietic neoplasms can generally follow the descriptions and diagnostic criteria employed by those of skill in the art (see, e.g., International Classification of Disease and Related Health Problems (ICD 10), World Health Organization (2003)). Hematopoietic neoplasms can also be characterized based on the molecular features, such as cell surface markers and gene expression profiles, cell phenotype exhibited by the aberrant cells, and/or chromosomal aberrations (e.g., deletions, translocations, insertions, etc.) characteristic of certain hematopoietic neoplasms, such as the Philadelphia chromosome found in chronic myelogenous leukemia. Other classifications include National Cancer Institute Working Formulation (Cancer, 1982, 49:2112-2135) and Revised European-American Lymphoma Classification (REAL).

[0030] The term “hematopoietic neoplasm” includes, but is not limited to, acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, Hodgkin’s disease, non-Hodgkin’s lymphoma, multiple myeloma, and myeloplastic syndrome.

[0031] “Myeloid neoplasm” refers to proliferative disorder of cells of the myeloid lineage of hematopoiesis. Neoplasms can arise from hematopoietic stem cells, myeloid committed progenitor cells, precursor cells, and terminally differentiated cells. Myeloid neoplasms can be subdivided based on the phenotypic attributes of the aberrant cells or the differentiated state from which the abnormal cells arise. Subdivisions include, among others, myeloproliferative diseases, myelodysplastic/myeloproliferative diseases, myelodysplastic syndromes, acute myeloid leukemia, and acute biphenotypic leukemia.

[0032] “Lymphoid neoplasm” refers a proliferative disorder involving cells of the lymphoid lineage of hematopoiesis. Lymphoid neoplasms can arise from hematopoietic stem cells as well as lymphoid committed progenitor cells, precursor cells, and terminally differentiated cells. These neoplasms can be subdivided based on the phenotypic attributes of the aberrant cells or the differentiated state from which the abnormal cells arise. Subdivisions include, among others, B cell neoplasms, T cell neoplasms, NK cell neoplasms, and Hodgkin’s lymphoma. Committed progenitor cells of the lymphoid lineage develop into the B cell pathway, T cell pathway, or the non-T/B cell pathway. Similar to the myeloid lineage, an additional lymphoid pathway appears to give rise to dendritic cells involved in antigen presentation. The B cell progenitor cell develops into a precursor B cell (pre-B), which differentiates into B cells responsible for producing immunoglobulins. Progenitor cells of the T cell lineage differentiate into precursor T cells (pre-T) that, based on the influence of certain cytokines, develop into cytotoxic or helper/suppressor T cells involved in cell mediated immunity. Non-T/B cell pathway leads to generation of natural killer (NK) cells.

[0033] The term “hematopoietic neoplasm” includes, but is not limited to, acute lymphoblastic leukemia (ALL), acute

myelogenous leukemia (AML), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, and myeloplastic syndrome.

[0034] As used herein, the term "pharmaceutically acceptable salt" includes salts that are physiologically tolerated by a subject. Such salts are typically prepared from an inorganic and/or organic acid. Examples of suitable inorganic acids include, but are not limited to, hydrochloric, hydrobromic, hydroiodic, nitric, sulfuric, and phosphoric acid. Organic acids may be aliphatic, aromatic, carboxylic, and/or sulfonic acids. Suitable organic acids include, but are not limited to, formic, acetic, propionic, succinic, camphorsulfonic, citric, fumaric, gluconic, lactic, malic, mucic, tartaric, para-toluenesulfonic, glycolic, glucuronic, maleic, furoic, glutamic, benzoic, anthranilic, salicylic, phenylacetic, mandelic, pamoic, methanesulfonic, ethanesulfonic, pantothenic, benzenesulfonic (besylate), stearic, sulfanilic, alginic, galacturonic, and the like. Other pharmaceutically acceptable salts include alkali metal cations (such as Na, K, Li), alkali earth metal salts (such as Mg or Ca), or organic amine salts (such as those disclosed in PCT International Application Nos. WO 02/22626 or WO 00/48606 and U.S. Pat. Nos. 6,855,702 and 6,670,344, which are incorporated herein by reference in their entireties). Particularly preferred salts include organic amine salts such tromethamine (TRIS) and amino acid salts such as histidine. Other exemplary salts that can be synthesized using the methods of the invention include those described in U.S. Pat. No. 7,018,987, which is incorporated by reference herein.

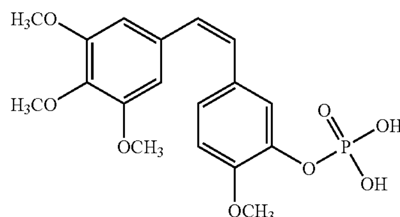
B. Methods of Treating Hematopoietic Neoplasms

[0035] Adhesion of leukemic cells to vascular cells may confer resistance to chemotherapeutic agents. Therefore, disruption of leukemic cell cytoskeletal stability and interference with vascular cell interactions should promote leukemic cell death. Indeed, as disclosed in greater detail below, low and non-toxic doses of combretastatin-A4 phosphate (CA4P) inhibit leukemic cell proliferation in vitro and induce mitotic arrest and cell death. Treatment of acute myeloid leukemias (AMLs) with CA4P leads to disruption of mitochondrial membrane potential, release of pro-apoptotic mitochondrial membrane proteins (MMPs) and DNA fragmentation, resulting in cell death in part through a caspase-dependent manner. In addition, CA4P rapidly increases intracellular reactive oxygen species (ROS), and antioxidant treatment imparts partial protection from cell death, suggesting that ROS accumulation contributes to CA4P-induced cytotoxicity in AML. In vivo, CA4P inhibits proliferation and circulation of leukemic cells and diminishes the extent of peri-vascular leukemic infiltrates, thereby prolonging the survival of xenotransplanted mice, without inducing hematological toxicity. CA4P decreases the interaction of leukemic cells with neo-vessels by down-regulating the expression of adhesion molecule, VCAM-1, thereby augmenting leukemic cell death. These data suggest that, CA4P target both circulating and vascular-adherent leukemic cells through mitochondrial damage and down-regulation of VCAM-1, without incurring hematological toxicities. As such, combretastatin A-4 phosphate provides for an effective means to treat refractory organ-infiltrating leukemias.

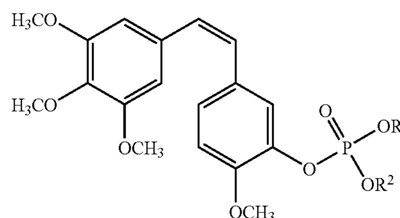
[0036] Accordingly, one aspect of the present invention provides a method of treating a hematopoietic neoplasm, the method comprising administering a therapeutically effective amount of combretastatin A-4 phosphate, or a pharmaceutically acceptable salt thereof, to a mammal suffering from a hematopoietic neoplasm. Preferably the pharmaceutically acceptable salt is a tromethamine salt of combretastatin A-4 phosphate.

[0037] Derived from the South African tree *Combreturn caffrum*, combretastatins were initially identified in the 1980's as potent inhibitors of tubulin polymerization. Combretastatin A-4 has been shown to bind a site at or near the colchicine binding site on tubulin with high affinity. In vitro studies clearly demonstrated that combretastatins are potent cytotoxic agents against a diverse spectrum of tumor cell types in culture. Phosphate prodrugs of combretastatin A-4 were subsequently developed to combat problems with aqueous insolubility. Surprisingly, CA4P has also been shown to cause a rapid and acute shutdown of the blood flow to tumor tissue that is separate and distinct from the anti-proliferative effects of the agents on tumor cells themselves. A number of studies have shown that combretastatins cause extensive shutdown of blood flow within the tumor microvasculature, leading to secondary tumor cell death (Dark et al., *Cancer Res.,* 57: 1829-34, (1997); Chaplin et al., *Anticancer Res.,* 19: 189-96, (1999); Hill et al., *Anticancer Res.,* 22(3):1453-8 (2002); Holwell et al., *Anticancer Res.,* 22(2A):707-11, (2002). Blood flow to normal tissues is generally far less affected by CA4P than blood flow to tumors, although blood flow to some organs, such as spleen, skin, skeletal muscle and brain, can be inhibited (Tozer et al., *Cancer Res.,* 59: 1626-34 (1999)).

[0038] As used herein, the term "combretastatin A-4 phosphate" denotes a compound of the Formula I:



[0039] One implementation comprises use of a compound of Formula II,



[0040] wherein

[0041] each OR^1 and OR^2 independently is selected from OH, $—O^-QH^+$ and $—O^-M^+$, wherein M^+ is a monovalent or divalent metal cation, and Q is:

[0042] a) an amino acid containing at least two nitrogen atoms where one of the nitrogen atoms, together with a proton, forms a quaternary ammonium cation QH^+; or

[0043] b) an organic amine containing at least one nitrogen atom which, together with a proton, forms a quaternary ammonium cation, QH^+. In another implementation, wherein one of OR^1 and OR^2 is hydroxyl, and the other is —O^-QH^+ where Q is L-histidine. In another embodiment, the combretastatin A-4 phosphate salt is a compound of Formula I, wherein one of OR^1 and OR^2 is hydroxyl and the other is —O^-QH^+ and Q is tris(hydroxymethyl)amino methane (tromethamine or "TRIS").

[0044] Another implementation comprises use of a compound of Formula II, wherein R^1 and R^2 are O^-M^+, wherein each M^+ independently is an aliphatic organic amine, an alkali metal, a transition metal, a heteroarylene, a heterocyclyl, a nucleoside, a nucleotide, an alkaloid, an amino sugar, an amino nitrile, or an nitrogenous antibiotic.

[0045] Yet another implementation comprises use of a compound of Formula II, wherein R^1 and R^2 are O^-M^+ and each M^+, independently, is sodium, TRIS, histidine, ethanolamine, diethanolamine, ethylenediamine, diethylamine, triethanolamine, glucamine, N-methylglucamine, ethylenediamine, 2-(4-imidazolyl)-ethylamine, choline, or hydrabamine. In a preferred implementation each M^+ is sodium.

[0046] The method of the invention can further comprise co-administering a chemotherapeutic agent, such a cytosine arabinoside (Ara-C), to the subject. "Co-administration" or "co-administering" can be in the form of a single formulation (combining, for example, CA4P and a Ara-C with pharmaceutically acceptable excipients, optionally segregating the two active ingredients in different excipient mixtures designed to independently control their respective release rates and durations) or by independent administration of separate formulations containing the active agents. "Co-administration" further includes concurrent administration (e.g. administration of CA4P and a Ara-C at the same time) and time varied administration (administration of CA4P at a time different from that of the Ara-C), as long as both the combretastatin A-4 phosphate, or a pharmaceutically acceptable salt thereof, and the chemotherapeutic agent, e.g., Ara-C, are present in the body in therapeutically effective concentrations during at least partially overlapping times. In preferred implementations the chemotherapeutic agent is Ara-C, etoposide, thioguanine or cyclophosphamide.

[0047] A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN® (cyclophosphamide); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gamma11 and calicheamicin omega11 (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL®) and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate, gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an epothilone, and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine (Ara-C), dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., paclitaxel (TAXOL®), albumin-engineered nanoparticle formulation of paclitaxel (ABRAXANE™), and doxetaxel (TAXOTERE®); chloranbucil; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovovin; vinorelbine (NAVELBINE®); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and

prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovovin.

[0048] As is well-known in the art, solid tumors are quite distinct from non-solid tumors, such as those found in hematopoietic-related cancers. Examples of non-solid tumors include leukemias, such as myeloid leukemias and lymphoid leukemias, myelomas, and lymphomas. In some implementations, the non-solid tumor cell is a hematopoietic neoplasm, which is aberrant growth of cells of the hematopoietic system. Hematopoietic malignancies can have its origins in pluripotent stem cells, multipotent progenitor cells, oligopotent committed progenitor cells, precursor cells, and terminally differentiated cells involved in hematopoiesis. Some hematological malignancies are believed to arise from hematopoietic stem cells, which have the ability for self renewal. For instance, cells capable of developing specific subtypes of acute myeloid leukemia (AML) upon transplantation display the cell surface markers of hematopoietic stem cells, implicating hematopoietic stem cells as the source of leukemic cells. Although hematopoietic neoplasms often originate from stem cells, committed progenitor cells or more terminally differentiated cells of a developmental lineage can also be the source of some leukemias. For example, forced expression of the fusion protein Bcr/Abl (associated with chronic myelogenous leukemia) in common myeloid progenitor or granulocyte/macrophage progenitor cells produces a leukemic-like condition. In a preferred implementation, the hematopoietic malignancy treated by the method of the invention is acute lymphoblastic leukemia (ALL) or acute myelogenous leukemia (AML).

[0049] Hematopoietic neoplasms differ from solid tumors in being capable of circulating and having access to various organs through interaction with activated vascular cells. Indeed, some hematopoietic neoplasms may adhere to vascular cells, establishing perivascular infiltrates, and as such may be endowed with a unique mechanism of resistance to chemotherapy. Both circulating and vascular-adherent hematopoietic neoplasms require cytoskeletal stability to maintain mitochondrial and cellular function and avoid cell death. Low and non-toxic doses of combretastatin A-4 phosphate can selectively induce apoptosis of circulating and vascular-bound leukemic cells, leading to cell death. This induction of apoptosis occurs by a caspase-dependent as well as ROS-mediated mitochondrial damage. Thus, combretastatin A-4 phosphate is effective in treating hematopoietic neoplasms, as demonstrating by its ability to target hematopoietic neoplasms in vitro and in vivo and to eradicate circulating, marrow- and organ-resident vascular-adherent hematopoietic neoplasms.

[0050] In some implementations, the hematopoietic neoplasm treated is a lymphoid neoplasm, where the abnormal cells are derived from and/or display the characteristic phenotype of cells of the lymphoid lineage. Lymphoid neoplasms can be subdivided into B-cell neoplasms, T and NK-cell neoplasms, and Hodgkin's lymphoma. B-cell neoplasms can be further subdivided into precursor B-cell neoplasm and mature/peripheral B-cell neoplasm. Exemplary B-cell neoplasms are precursor B-lymphoblastic leukemia/lymphoma (precursor B-cell acute lymphoblastic leukemia) while exemplary mature/peripheral B-cell neoplasms are B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone B-cell lymphoma, hairy cell leukemia, plasma cell myeloma/plasmacytoma, extranodal marginal zone B-cell lymphoma of MALT type, nodal marginal zone B-cell lymphoma, follicular lymphoma, mantle-cell lymphoma, diffuse large B-cell lymphoma, mediastinal large B-cell lymphoma, primary effusion lymphoma, and Burkitt's lymphoma/Burkitt cell leukemia. T-cell and NK-cell neoplasms are further subdivided into precursor T-cell neoplasm and mature (peripheral) T-cell neoplasms. Exemplary precursor T-cell neoplasm is precursor T-lymphoblastic lymphoma/leukemia (precursor T-cell acute lymphoblastic leukemia) while exemplary mature (peripheral) T-cell neoplasms are T-cell prolymphocytic leukemia T-cell granular lymphocytic leukemia, aggressive NK-cell leukemia, adult T-cell lymphoma/leukemia (HTLV-1), extranodal NK/T-cell lymphoma, nasal type, enteropathy-type T-cell lymphoma, hepatosplenic gamma-delta T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma, Mycosis fungoides/Sezary syndrome, Anaplastic large-cell lymphoma, T/null cell, primary cutaneous type, Peripheral T-cell lymphoma, not otherwise characterized, Angioimmunoblastic T-cell lymphoma, Anaplastic large-cell lymphoma, T/null cell, primary systemic type. The third member of lymphoid neoplasms is Hodgkin's lymphoma, also referred to as Hodgkin's disease. Exemplary diagnosis of this class that can be treated with the compounds include, among others, nodular lymphocyte-predominant Hodgkin's lymphoma, and various classical forms of Hodgkin's disease, exemplary members of which are Nodular sclerosis Hodgkin's lymphoma (grades 1 and 2), Lymphocyte-rich classical Hodgkin's lymphoma, Mixed cellularity Hodgkin's lymphoma, and Lymphocyte depletion Hodgkin's lymphoma. In various implementations, any of the lymphoid neoplasms can be treated with the combretastatin A-4 phosphate, or a pharmaceutically acceptable salt thereof.

[0051] In some implementations, the hematopoietic neoplasm treated is a myeloid neoplasm. This group comprises a large class of cell proliferative disorders involving or displaying the characteristic phenotype of the cells of the myeloid lineage. Myeloid neoplasms can be subdivided into myeloproliferative diseases, myelodysplastic/myeloproliferative diseases, myelodysplastic syndromes, and acute myeloid leukemias. Exemplary myeloproliferative diseases are chronic myelogenous leukemia (e.g., Philadelphia chromosome positive (t(9;22)(qq34;q11)), chronic neutrophilic leukemia, chronic eosinophilic leukemia/hypereosinophilic syndrome, chronic idiopathic myelofibrosis, polycythemia vera, and essential thrombocythemia. Exemplary myelodysplastic/myeloproliferative diseases are chronic myelomonocytic leukemia, atypical chronic myelogenous leukemia, and juvenile myelomonocytic leukemia. Exemplary myelodysplastic syndromes are refractory anemia, with ringed sideroblasts and without ringed sideroblasts, refractory cytopenia (myelodysplastic syndrome) with multilineage dysplasia, refractory anemia (myelodysplastic syndrome) with excess blasts, 5q-syndrome, and myelodysplastic syndrome. In various implementations, any of the myeloid neoplasms can be treated with combretastatin A-4 phosphate, or a pharmaceutically acceptable salt thereof.

[0052] In some implementations, combretastatin A-4 phosphate, or a pharmaceutically acceptable salt thereof, can be used to treat acute myeloid leukemias (AML), which represent a large class of myeloid neoplasms having its own subdivision of disorders. These subdivisions include, among others, AMLs with recurrent cytogenetic translocations, AML with multilineage dysplasia, and other AML not otherwise

categorized. Exemplary AMLs with recurrent cytogenetic translocations include, among others, AML with t(8;21)(q22; q22), AML1 (CBF-alpha)/ETO, Acute promyelocytic leukemia (AML with t(15;17)(q22;q11-12) and variants, PML/RAR-alpha), AML with abnormal bone marrow eosinophils (inv(16)(p13q22) or t(16;16)(p13;q11), CBFb/MYH11X), and AML with 11q23 (MLL) abnormalities. Exemplary AML with multilineage dysplasia are those that are associated with or without prior myelodysplastic syndrome. Other acute myeloid leukemias not classified within any definable group include, AML minimally differentiated, AML without maturation, AML with maturation, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroid leukemia, acute megakaryocytic leukemia, acute basophilic leukemia, and acute panmyelosis with myelofibrosis.

[0053] One aspect of the invention is a pharmaceutical composition useful for treating a hematopoietic neoplasm in a warm-blooded animal, which composition comprises combretastatin A-4 phosphate, or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable excipient. The composition is prepared in accordance with known formulation techniques to provide a composition suitable for oral, topical, transdermal, rectal, by inhalation, parenteral (intravenous, intramuscular, or intraperitoneal) administration, and the like. Detailed guidance for preparing compositions of the invention can be found by reference to the 18th or 19th Edition of Remington's Pharmaceutical Sciences, Published by the Mack Publishing Co., Easton, Pa. 18040. In certain implementations, the pharmaceutical composition further comprises a chemotherapeutic agent, such as Ara-C, etoposide, thioguanine or cyclophosphamide.

[0054] Unit doses or multiple dose forms are contemplated, each offering advantages in certain clinical settings. The unit dose would contain a predetermined quantity of active compound calculated to produce the desired effect(s) in the setting of treating cancer. The multiple dose form may be particularly useful when multiples of single doses, or fractional doses, are required to achieve the desired ends. Either of these dosing forms may have specifications that are dictated by or directly dependent upon the unique characteristic of the particular compound, the particular therapeutic effect to be achieved, and any limitations inherent in the art of preparing the particular compound for treatment of cancer.

[0055] A unit dose will contain a therapeutically effective amount sufficient to treat a hematopoietic neoplasm in a subject and may contain from about 1.0 to 1000 mg of compound, for example about 50 to 500 mg.

[0056] The combretastatin A-4 phosphate, or a pharmaceutically acceptable salt thereof, preferably is administered parenterally, e.g., intravenously, intramuscularly, intravenously, subcutaneously, or intraperitoneally. The carrier or excipient or excipient mixture can be a solvent or a dispersive medium containing, for example, various polar or non-polar solvents, suitable mixtures thereof, or oils. As used herein "carrier" or "excipient" means a pharmaceutically acceptable carrier or excipient and includes any and all solvents, dispersive agents or media, coating(s), antimicrobial agents, iso/hypo/hypertonic agents, absorption-modifying agents, and the like. The use of such substances and the agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use in therapeutic compositions is contemplated. Moreover, other or supplementary active ingredients can also be incorporated into the final composition.

[0057] Solutions of the compound may be prepared in suitable diluents such as water, ethanol, glycerol, liquid polyethylene glycol(s), various oils, and/or mixtures thereof, and others known to those skilled in the art.

[0058] The pharmaceutical forms suitable for injectable use include sterile solutions, dispersions, emulsions, and sterile powders. The final form must be stable under conditions of manufacture and storage. Furthermore, the final pharmaceutical form must be protected against contamination and must, therefore, be able to inhibit the growth of microorganisms such as bacteria or fungi. A single intravenous or intraperitoneal dose can be administered. Alternatively, a slow long term infusion or multiple short term daily infusions may be utilized, typically lasting from 1 to 8 days. Alternate day or dosing once every several days may also be utilized.

[0059] Sterile, injectable solutions are prepared by incorporating a compound in the required amount into one or more appropriate solvents to which other ingredients, listed above or known to those skilled in the art, may be added as required. Sterile injectable solutions are prepared by incorporating the compound in the required amount in the appropriate solvent with various other ingredients as required. Sterilizing procedures, such as filtration, then follow. Typically, dispersions are made by incorporating the compound into a sterile vehicle which also contains the dispersion medium and the required other ingredients as indicated above. In the case of a sterile powder, the preferred methods include vacuum drying or freeze drying to which any required ingredients are added.

[0060] In all cases the final form, as noted, must be sterile and must also be able to pass readily through an injection device such as a hollow needle. The proper viscosity may be achieved and maintained by the proper choice of solvents or excipients. Moreover, the use of molecular or particulate coatings such as lecithin, the proper selection of particle size in dispersions, or the use of materials with surfactant properties may be utilized.

[0061] Prevention or inhibition of growth of microorganisms may be achieved through the addition of one or more antimicrobial agents such as chlorobutanol, ascorbic acid, parabens, thermerosal, or the like. It may also be preferable to include agents that alter the tonicity such as sugars or salts.

[0062] Another aspect of this invention is a method for treating a hematopoietic neoplasm in a warm-blooded animal, which method comprises administering a therapeutically effective amount of combretastatin A-4 phosphate, or a pharmaceutically acceptable salt thereof. The combretastatin A-4 phosphate, or a pharmaceutically acceptable salt thereof, can be administered to an appropriate subject in a therapeutically effective dose by a medically acceptable route of administration such as orally, parentally (e.g., intramuscularly, intravenously, subcutaneously, interperitoneally), transdermally, rectally, by inhalation and the like.

[0063] With mammals, including humans, the effective amounts can be administered on the basis of body surface area. The interrelationship of dosages varies for animals of various sizes and species, and for humans (based on mg/m^2 of body surface) is described by E. J. Freireich et al., Cancer Chemother. Rep., 50(4):219 (1966). Body surface area may be approximately determined from the height and weight of an individual (see, e.g., Scientific Tables, Geigy Pharmaceuticals, Ardsley, N.Y. pp. 537-538 (1970)). A suitable dose

range is from 1 to 1000 mg of equivalent per m^2 body surface area of a compound of the invention, for instance from 50 to 500 mg/m^2.

[0064] Another important feature of the method provided by the present invention relates to the relatively low apparent overall toxicity of the derivatives administered in accordance with the teachings herein. Overall toxicity can be judged using various criteria. For example, loss of body weight in a subject over 10% of the initially recorded body weight (i.e., before treatment) can be considered as one sign of toxicity. In addition, loss of overall mobility and activity and signs of diarrhea or cystitis in a subject can also be interpreted as evidence of toxicity.

VI. EXAMPLES

A. Example 1

Combretastatin A4 Phosphate (CA4P) Inhibits Leukemic Cell Proliferation

[0065] Leukemic cells were seeded at 1×10^5 cells/ml in X-vivo medium (Bio-Whittaker, MA) with 5% FBS and CA4P or pre-incubated with the PARP-inhibitor DPQ or caspase-inhibitors Z-VAD-fmk and Q-VD-OPh (R&D, Minneapolis, Minn.). A panel of leukemic cells was incubated with CA4P at the different concentrations indicated in FIG. **1**, and viable cells were counted after 48 hours using trypan blue exclusion. Results of four experiments in duplicate are expressed as the ratio of the percentage of viable cells/control±SEM (*p<0.05 as compared with CA4P-untreated cells; n=4). After incubation for 48 hours, cells were counted using trypan-blue exclusion. CA4P at concentrations as low as 1 nM initiated cell death of non-adherent, anchorage-independent AML cells in vitro, with the IC_{50} ranging from 2.5 to 5 nM (FIG. **1**). The majority of the AML cell lines tested was sensitive to CA4P at a concentration of 2.5 nM or less. All leukemic cell lines, as well as a recently established primary leukemic cell line (R81) 16 were sensitive at low doses of CA4P (<10 nM).

B. Example 2

CA4P Causes Cell Cycle Arrest in G2/M Phase

[0066] CA4P induces G2/M arrest and cell death, as evidenced by increase in the sub-G0/G1 peak (FIG. **2**). Leukemic cells were seeded at 10^5 per ml in X-vivo supplemented with 5% FBS and then incubated with CA4P. KG1a leukemic cells were treated with CA4P at 0, 5 and 10 nM concentrations. After incubation for 48 hours, apoptotic cells were quantified by ApoAlert Annexin-V-fluorescine isothiocyanate (FITC) propidium iodide (PI) Apoptosis Kit (BD) using a Coulter Elite flow cytometer. In CA4P-treated cells, cell cycle analysis with propidium iodine (PI) showed G_2/M arrest and evidence of DNA fragmentation (sub G_0-phase) at 48 hours.

C. Example 3

Combretastatin A-4 Phosphate Causes DNA Fragmentation and Morphological Evidence of Mitotic Catastrophe

[0067] DNA damage in CA4P-incubated leukemic cells was assessed by comet assay. The concept behind this assay is based upon the ability of denatured, cleaved DNA fragments to migrate out of the cell under the influence of an electric field, whereas undamaged DNA migrates more slowly and remains within the confines of the nucleus. Evaluation of the DNA "comet" tail shape and migration pattern allows for assessment of DNA damage. Results were expressed as the percentage of cells with a comet tail in 100 randomly selected, non-overlapping cells visualized by standard light microscopy. Quantification of the number of leukemic cells displaying a comet tail strongly increased after CA4P treatment (FIG. **3**), consistent with CA4P-induced DNA damage. Results of three experiments in duplicate are expressed as the mean of the number of cells with a comet tail (%)±SEM (*p<0.05 as compared with CA4P-untreated cells; n=3).

D. Example 4

CA4P Induces Cell Death Through Apoptosis without Evidence of Necrosis

[0068] CA4P-treated AML cells were subjected to Annexin-V/propidium iodide (PI) staining and quantification by flow cytometry. Combretastatin A-4 phosphate (CA4P) induces caspase-independent apoptosis in leukemic cells. CA4P induces apoptosis of leukemic cells. Leukemic cells were treated with or without CA4P for 48 hours and the percentage of apoptotic leukemic cells was determined by Annexin V and propidium iodide staining using flow cytometry. Results are representative of three independent experiments. In contrast to endothelial cells, CA4P induced phosphatidylserine externalization in all leukemic cell lines tested, suggesting that CA4P promotes leukemic cell death through apoptosis (FIG. **4**). In the majority of AML cell lines tested, CA4P at a concentration of 5 nM induced phosphatidylserine externalization (Annexin V+) in more than 50% of the leukemic cells. Only a small number of PI+, Annexin V(−) cells were detected, suggesting that CA4P-mediated cell death is apoptotic rather than necrotic.

[0069] Nonetheless, to rule out the possibility that CA4P may trigger necrotic cell death in subsets of the leukemic cells, the release of nuclear HMG1 protein into the culture supernatant, which has been described as a sensitive and highly specific marker for necrotic cell death (Scaffidi, et al. 2002. *Nature* 418, 191-5), was quantified. For detection of HMG1 protein, KG1a leukemic cells (1×106 in 2 ml X-vivo/5% FBS) were incubated with 100 μM staurosporine to induce apoptosis, freeze-thawed three times to induce necrosis, or treated with 20 mM CA4P for 24 hours. Cells were spun down, pelleted and the supernatant subjected to Western blot for HMG135. Western blot shows release of pelleted (P) nuclear HMG1 protein into the supernatant (S) in necrotic (freeze-thaw), but not apoptotic (staurosporine-treated) or CA4P-treated KG1a leukemic cells (data not shown). Identical results were obtained in three independent experiments.

E. Example 5

CA4P-Induced Cell Death is Partially Caspase-Dependent

[0070] Both Z-VAD-fmk and Q-VD, two potent general caspase inhibitors, blocked CA4P-induced caspase-3 activation, but only partially affected CA4P-induced hematopoietic neoplasm death, (FIG. **5**). Leukemic cells were treated with or without CA4P for 48 hours in presence of caspase inhibitor Q-VD and the percentage of apoptotic leukemic cells was determined by Annexin V and propidium iodide staining using flow cytometry. Percentage of alive Annexin V(−)/PI(−) cells was plotted. Results are average of three independent

experiments (*p=0.008 as compared with CA4P-treated cells). Quantification of apoptosis by Annexin V/PI staining showed that only 33% of the CA4P induced apoptosis was blocked by Q-VD in HL60 cells (FIG. **5**). Z-VAD-fmk had no effect (data not shown). These data suggest that CA4P induces apoptosis of hematopoietic neoplasms through caspase-dependent and non-caspase-dependent pathways.

F. Example 6

CA4P Decreases Mitochondrial Transmembrane Potential

[0071] To determine mitochondrial membrane potential, leukemic cells incubated with CA4P for 48 hours were harvested, and incubated for 10 minutes at 37° C. in serum-free culture medium at a concentration of 2×105 cells/ml with 20 nM of 3,30-dihexyloxacarbocyanine (DiOC6(3), Molecular Probes), a cell-permeant, green-fluorescent, lipophilic dye that is selective for the mitochondria of viable cells. Cells were collected by centrifugation and analyzed by flow cytometry.

[0072] The pattern of $DiOC_6(3)$ fluorescence taken up by control leukemic cells showed cell populations with bright fluorescence, representing cells with intact high MTP (FIG. **6**). Results are representative of three independent experiments. In contrast, the amount of $DiOC_6(3)$ dye taken up in CA4P-treated leukemic cells was strongly decreased. The percentage of cells with fluorescence below control ranged from 12 to 89% of total cells, and for each cell line tested, the results correlated well with the extent of Annexin-V positivity. These data indicated that CA4P-induced leukemic cell death is possibly mediated through alteration of mitochondrial permeability.

[0073] Mitochondrial damage may result in the release of pro-apoptotic mitochondrial membrane proteins (MMPs) such as cytochrome c, SMAC/diablo and ARTS. By immunofluorescence, we observed the release of cytochrome c and ARTS from mitochondria in leukemic cells after CA4P exposure (data not shown), consistent with mitochondrial destabilization. Cytochrome c and ARTS were detected using mAb clone 6H2.B4 (1:100, BD Pharmingen) and polyclonal-antibody A3720 (1:50, Sigma), both followed by AlexaFluor 488 conjugated secondary antibody (1:200, Molecular Probes) and analyzed by confocal microscopy.

G. Example 7

Reactive Oxygen Species (ROS) Accumulation

[0074] Leukemic cells were treated with 5 nM (HL60, R81, U937) or 10 nM (KG1a, THP-1) for 48 hours with the ascorbic acid concentrations indicated and viable cells determined by trypan blue exclusion. Results of three experiments in triplicate are expressed as percentage of viable cells compared to control±SEM (*p<0.05 as compared with CA4P-untreated cells; n=3). Co-incubation of leukemic cells with the antioxidant ROS scavenger, ascorbic acid (AA), led to a partial decrease in CA4P-mediated cell death (FIG. **7**).

[0075] In addition, rapid accumulation of superoxide anions after CA4P exposure, as evidenced by an increase in intracellular fluorescence after H_2DCFDA loading and reversal of ROS accumulation by ascorbic acid (FIG. **8**), was also detected. Intracellular ROS were detected as previously described (Krejsa & Schieven, *Methods Mol Biol* 99:35-47 (2000)). Briefly, leukemic cells were loaded with 2 μM H2DCFDA (Molecular Probes) in assay buffer (RPMI with 10 mM HEPES) for 30 min. at 37° C. and mean fluorescent intensity was measured by flow cytometry. Results are shown from triplicate measurements (±SEM) and are representative of three independent experiments (*p<0.05 as compared with untreated cells; n=3).

[0076] HL-60 cells were treated with or without CA4P for 48 hours in presence of another ROS scavenger, deferoxamine (DFX), and caspase inhibitor Q-VD and the percentage of apoptotic leukemic cells was determined by Annexin V and propidium iodide staining using flow cytometry. Results are average of three independent experiments (*p=0.01 as compared with CA4P-treated cells, *p=0.04 as compared to CA4P+Q-VD treated cells). DFX also slightly inhibited CA4P-induced cell death and had an additive effect with caspase inhibitor Q-VD in HL60 cells, inhibiting up to 71% of CA4P-induced cell death (FIG. **9**). Taken together, these data indicate that CA4P induces cell death in part through a caspase-dependent as well as in part through a non-caspase dependent cell pathway, by accumulation of ROS as a result of tubulin-destabilization and disruption of the mitochondrial respiratory chain.

H. Example 8

Subcutaneous In Vivo Leukemia Model

[0077] HL60 (5×10^6 cells) were injected subcutaneously into the dorsa of seven-week-old NOD-SCID mice (Jackson Laboratory). When mice bore a tumor (i.e. after 12 days), 4 experimental groups were randomized, each with 9 animals. Daily treatment was initiated at this time: the CA4P groups were subjected to intraperitoneal injection of CA4P at 10, 25 and 50 mg/kg body weight, and the control group received PBS. After a 3-day treatment, animals were sacrificed, tumors removed and then subjected to immunohistochemical analysis. Tumors were embedded in paraffin, serially sectioned, and stained with hematoxylin and eosin for histological analysis. Cell death was assessed by TUNEL assay. Cell death within paraffin-embedded tumor sections was detected by TUNEL reaction (Roche Diagnostics). The detection of cell death in this assay relies on the detection of free 3'OH DNA ends. Positive signal was revealed by fast red and tumor sections analyzed by light microscopy after hematoxylin counterstain.

[0078] Tumors from mice treated with CA4P for 3 days were softer and hemorrhagic (particularly when treated with 50 mg/kg) than those observed in the control group. Tumor sections of the control untreated mice showed large areas of viable HL60 cells without significant necrosis or fibrosis. In contrast, all tumors in CA4P treated mice were largely necrotic. The control tumor sections were negative for TUNEL reaction. In sharp contrast, most of the leukemic cells were non-viable following treatment with low to high doses of CA4P (data not shown). The extent of intra-tumor vascularization within the different groups was assessed by immunostaining for the endothelial-specific antigen MECA-32. Control tumors exhibited abundant number of vessels, whereas CA4P-treated tumors showed only a very small number of neo-vessels (FIG. **10**, p<0.05, n=5). The neo-vessel density was evaluated by microscopic counting of 5 fields at 10× magnification, and presented as mean number of microvessels per microscopic field±SEM (*p<0.05 as compared with the PBS control group; n=5). The decrease in intra-tumor vascularization induced by CA4P was dose

dependent (54±13%, 68±6% and 90±3% decrease for 10, 25 and 50 mg/kg, respectively). These data suggest that targeting leukemic xenografts is associated with tumor regression and inhibition of neo-angiogenesis.

I. Example 9

Systemic In Vivo Leukemia Model

[0079] NOD-SCID mice were intravenously inoculated with 1×10^7 GFP+HL60 cells. The HL60 cells were labeled with green fluorescent protein (GFP) by a lentiviral construct, and inoculated systemically through tail vein injection. Three days after inoculation, mice were divided into 2 groups of five mice. One group was treated every other day with PBS (control) and the second group received 25 mg/kg CA4P every other day. Each experiment was done three times. At day 30 after the start of the experiment, two mice from each group were killed, and their organs (spleen, liver and lung), peripheral blood and marrow of surgically removed femurs were collected, and analyzed for the presence of human leukemic cells by flow cytometry. Single cell suspension was stained using phytoerythrin-labeled anti-human CD45 mAb (Pharmingen), and the percentage of double positive human CD45-PE and GFP cells was determined flow cytometer. The extent of GFP+HL60 cell infiltration was assessed by fluorescence microscopy. In a second set of experiments, GFP+U937 cells were used instead of HL60 cells, and the animals sacrificed after 30 days.

[0080] In the untreated control group, the mice survived for 32-40 days only and succumbed to systemic spread of leukemia. In contrast, CA4P treatment significantly increased survival of the intravenously xenotransplanted mice (FIG. **11**, $p<0.05$; n=5). This increase in survival was accompanied by a decrease in circulating leukemic cells observed 30 days after leukemic cell injection, as assessed by flow cytometry determination of double positive cells for human CD45 and GFP (0.9% for control vs. 0.2% for CA4P treatment, $p<0.05$, n=5) (FIG. **12**). Thirty days after xenotransplantation of GFP+HL60 cell injection, the presence of GFP+HL60 cells in the peripheral blood and the different tissues of the mice was assessed by quantification of double positive GFP and human CD45 cells by flow cytometry. Remarkably, engraftment of leukemic cells in the bone marrow was completely eradicated in the CA4P-treated mice (FIG. **12**). In addition, there was no evidence of infiltration of GFP+HL60 cells in the spleen and the lung of CA4P-treated mice, whereas a small population (0.1%) of human CD45 and GFP positive cells was detected in the liver (FIG. **12**). In contrast, control mice had massive leukemic infiltrates in the spleen (5.2%), liver (2.6%) and lung (3.5%) (FIG. **12**). Concurrent histological analysis showed the presence of leukemic infiltrates in spleen, liver and lung sections of control mice, but only minimal foci of leukemic cells in the liver of CA4P-treated mice, confirming a drastic decrease in the amount of residual disease.

[0081] To confirm these results with a different leukemic cell line, the experiments were repeated using GFP-labeled U937 cells and harvested organs after 30 days. The results obtained were similar to the experiment using HL60 cells: in the treatment group, less than 0.05% GFP-positive U937 leukemic cells were detectable in the liver and bone marrow, whereas the control animals had significant leukemic organ infiltration present in the spleen, liver and lungs (data not shown). These data show that CA4P can efficiently block systemic hematopoietic neoplasm growth in vivo and inhibit organ-specific spread of hematopoietic neoplasms, apparently through disruption of hematopoietic neoplasm growth, migration and possibly interfering with the activation of vascular stromal cells.

J. Example 10

CA4P Modulates Cell Adhesion

[0082] CA4P may modulate leukemia-vascular interactions, thereby disrupting chemo-protected niches for leukemic cells. Indeed, CA4P treatment of leukemia xenografts significantly reduced expression of VCAM-1, a VLA4 ligand and key molecule in leukemia-stroma adhesion. NOD-SCID mice with subcutaneous HL60 AML tumors were treated with CA4P or PBS (untreated control). Immunofluorescence for VCAM-1 showed significantly decreased VCAM-1 expression in CA4P-treated tumors (data not shown).

[0083] Human umbilical vein endothelial cells (HUVECs) were activated with IL-1β (5 ng/ml) for 24 hours with CA4P added at concentrations from 0 to 5 nM. VCAM-1 expression was determined by flow cytometry with phytoerythrin-conjugated anti-CD106 (VCAM-1) mAb. Treatment of HUVECs with low, non-toxic (1 to 5 nM range) doses of CA4P significantly reduced expression of VCAM-1 (FIG. **13**), without inducing apoptosis.

[0084] To assess leukemic cell adhesion, 1×10^5 GFP+ HL60 or U937 cells in X-vivo/5% FBS were added per well. The percentage of GFP++adherent cells was quantified by fluorescent microscopy. To compare the resistance of vascular-adherent to non-adherent leukemic cells in co-culture, leukemic cells were seeded on either IL-1β-activated or non-activated HUVECs, with addition of CA4P. After 48 hours, GFP++leukemic cells were removed from the wells by trypsinization and quantified by fluorescence microscopy and flow cytometry. Treatment with CA4P led to a decreased attachment of HL60 and U937 AML cells to HUVECs in vitro (FIGS. **14**A and **14**B). The number of adherent cells is expressed as a percentage of total leukemic cells and representative of three independent experiments performed in triplicate SEM (*$p<0.05$ as compared to CA4P-untreated control).

[0085] In turn, decreased adhesion to HUVECs rendered the leukemic cells more sensitive to subsequent CA4P treatment (FIGS. **15**A and **15**B). Survival of GFP+U937 (E) and HL60 (F) leukemic cells co-cultured with HUVECs is expressed as a percentage of total cells. Adherent (i.e. attached to IL-1β activated HUVECs) and non-adherent leukemic cells (i.e. cultured with non-activated HUVECs) are depicted. Results are representative of three independent experiments performed in triplicate±SEM (*$p<0.05$). Taken together, these findings suggest that CA4P reduces expression of VCAM-1 on vascular cells, thereby increasing the chemosensitivity of hematopoietic neoplasms. Most notably, low CA4P concentrations reduced the expression of VCAM-1 without inducing endothelial cell death, suggesting that CA4P exerts an anti-leukemic effect by modulating adhesive function of the endothelial cells prior to its anti-angiogenic effect.

K. Example 11

CA4P has Minimal Bone Marrow Toxicity

[0086] Eight week-old sex matched CD1 mice (Jackson Laboratory, Bar Harbor, Me.) were treated with CA4P at 25

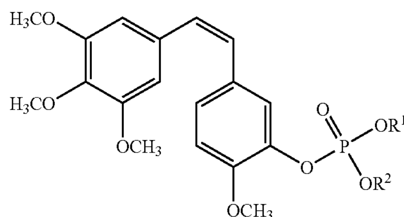
mg/kg subcutaneously every other day for 4 weeks. Serial complete blood counts were monitored using Bayer ADVIA 120 hematology analyzer. the mice so treated for 4 weeks displayed a slight decrease in WBC and absolute neutrophil count only, suggesting minimal marrow suppression.

[0087] In addition, human umbilical cord derived (CB) CD34+ cells cultured in vitro in the presence of kit-ligand (Stem Cell Factor) and CA4P for 48 h produced only minimal effect on cell viability as assayed by Annexin/PI staining. Cord blood CD34+ cells were isolated by magnetic procedure and cultured with recombinant human SCF (50 ng/ml, Peprotech) and CA4P for 48 h. Annexin/PI staining was then performed. CD34+ cells were also cultured in methylcellulose supplemented with cytokines and CA4P (Stem cell technologies, Vancouver, Canada) for 14 days. Colonies were scored. Moreover, CA4P did not impair colony-forming potential of CD34+ cells, demonstrating that CA4P at the concentrations used to target hematopoietic neoplasms has no major toxic effect on normal stem or progenitor cell function. As such, CA4P used as single agent can selectively target circulating and or tissue-resident hematopoietic neoplasms without incurring significant hematological toxicity.

We claim:

1. A method of treating a hematopoietic neoplasm comprising administering a therapeutically effective amount of combretastatin A-4 phosphate (CA4P), or a pharmaceutically acceptable salt thereof, to a subject having a hematological malignancy.

2. The method of claim **1**, wherein the combretastatin A-4 phosphate salt is a compound of the Formula II:



wherein

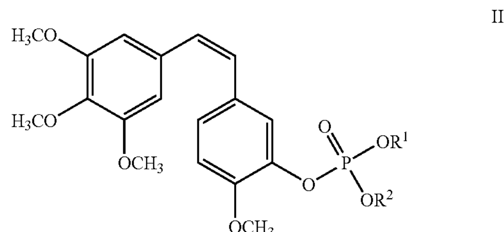
each OR^1 and OR^2 independently is selected from OH, —O^-QH^+ and —O^-M^+, wherein M^+ is a monovalent or divalent metal cation, and Q is:

a) an amino acid containing at least two nitrogen atoms where one of the nitrogen atoms, together with a proton, forms a quaternary ammonium cation QH^+; or

b) an organic amine containing at least one nitrogen atom which, together with a proton, forms a quaternary ammonium cation, QH^+.

3. The method of claim **2**, wherein one of OR^1 and OR^2 is hydroxyl and the other is —O^-QH^+ and Q is tromethamine or L-histidine.

4. The method of claim **1**, wherein the combretastatin A-4 phosphate salt is a compound of the Formula II:



wherein

each OR^1 and OR^2 independently is selected from OH, and —O^-M^+, wherein each M^+ independently is an aliphatic organic amine, an alkali metal, a transition metal, a heteroarylene, a heterocyclyl, a nucleoside, a nucleotide, an alkaloid, an amino sugar, an amino nitrile, or an nitrogenous antibiotic.

5. The method of claim **4**, wherein each M^+, independently, is selected from the group consisting of sodium, TRIS, histidine, ethanolamine, diethanolamine, ethylenediamine, diethylamine, triethanolamine, glucamine, N-methylglucamine, ethylenediamine, 2-(4-imidazolyl)-ethylamine, choline, and hydrabamine.

6. The method of claim **1**, wherein the hematopoietic neoplasm is a myeloid neoplasm.

7. The method of claim **6**, wherein the hematopoietic neoplasm is an acute myeloid leukemia (AML).

8. The method of claim **1**, wherein the hematopoietic neoplasm is a lymphoid neoplasm.

9. The method of claim **1**, wherein the hematopoietic neoplasm is a refractory organ-infiltrating leukemia.

10. The method of claim **1**, wherein the pharmaceutically acceptable salt is a tromethamine salt of combretastatin A-4 phosphate.

11. The method of claim **1**, further comprising co-administering a chemotherapeutic agent to the subject having a hematological malignancy.

12. The method of claim **11**, wherein the chemotherapeutic agent is Ara-C, etoposide, thioguanine or cyclophosphamide.

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