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(56) Fremdragne publikationer:
EP-A2- 0 276 051
WO-A1-02/056692
WO-A2-2004/078126
US-A1- 2002 119 951
US-A1- 2003 220 298
US-A1- 2006 035 868
US-A1- 2007 073 077
US-A1- 2007 149 476
LIANG FANG ET AL: "MZ3 induces apoptosis in human leukemia cells", CANCER CHEMOTHERAPY AND
PHARMACOLOGY, SPRINGER, BERLIN, DE, vol. 59, no. 3, 10 August 2006 (2006-08-10), pages 397-405,
XP019472996, ISSN: 1432-0843, DOI: DOI:10.1007/S00280-006-0294-6
NABHA S M ET AL: "Evaluation of combretastatin A-4 prodrug in a non-Hodgkin's lymphoma xenograft model:
Preclinical efficacy", ANTI-CANCER DRUGS 2001 GB LNKD- DOI:10.1097/00001813-200101000-00008, vol. 12,
no. 1, 2001, pages 57-63, XP009142168, ISSN: 0959-4973
NABHA S M ET AL: "EFFECTS OF COMBRETASTATIN A-4 PRODRUG AGAINST A PANEL OF MALIGNANT
HUMAN B-LYMPHOID CELL LINES", ANTI-CANCER DRUGS, LIPPINCOTT WILLIAMS & WILKINS, US; NL, vol.
11, no. 5, 1 June 2000 (2000-06-01), pages 385-392, XP009082802, ISSN: 0959-4973, DOI: DOI:10.1097/00001813-

Fortsættes ...

200006000-00009

Petit I; Karajannis M A; Vincent L; Young L; Butler J; Hooper A T; Shido K; Steller H; Chaplin D J; Feldman E; Rafii S: "The microtubule-targeting agent CA4P regresses leukemic xenografts by disrupting interaction with vascular cells and mitochondrial-dependent cell death", *Blood*, 16 November 2007 (2007-11-16), XP002613257, DOI: 10.1182/blood-2007-05-089219 Retrieved from the Internet: URL:<http://bloodjournal.hematologylibrary.org/cgi/reprint/blood-2007-05-089219v1> [retrieved on 2010-12-07]

BISHOP J F ET AL: "A randomized study of high-dose cytarabine in induction in acute myeloid leukemia", *BLOOD* 19960301 US, vol. 87, no. 5, 1 March 1996 (1996-03-01), pages 1710-1717, XP002613258, ISSN: 0006-4971

VARDIMAN ET AL: "The World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues: An overview with emphasis on the myeloid neoplasms", *CHEMICO-BIOLOGICAL INTERACTIONS*, ELSEVIER SCIENCE IRLAND, IR, vol. 184, no. 1-2, 19 March 2010 (2010-03-19), pages 16-20, XP026982147, ISSN: 0009-2797 [retrieved on 2009-10-24]

Description

I. Introduction

[0001] Cancer is a leading cause of death in the industrialized world and despite years of research, many types of cancer lack an effective therapeutic treatment. Although chemotherapy induces remission in the majority of adult patients with acute myeloid leukemia (AML), only a 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 vascular cells, interfering with the establishment of pro-leukemic niches in various organs and to eradicate resistant disease.

[0002] 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.

[0003] Combretastatin A-1, a novel tubulin-destabilizing agent, was isolated from the South African tree *Combretum caffrum*. Combretastatin A-1 binds to tubulin at the same site as colchicine, but with higher affinity. Its phosphate pro-drug, combretastatin A-1 phosphate (CA1dP) induces rapid microtubule depolymerization and vascular shutdown in subcutaneous solid tumors causing tumor necrosis at concentrations well below the maximum tolerated dose. Combretastatin A-1 comprises a single ortho-catechol moiety and is known to be capable of generating an enhanced antitumor response by forming reactive oxygen species (ROS) in the locality of the tumor in addition to selectively reducing the flow of blood to at least a portion of a tumor. This results in both direct inhibition of the proliferation of tumor cells, and tumor selective induction of hypoxia and subsequent necrosis in a portion of the tumor tissue without substantial necrosis of adjoining non-tumor tissue. Other catechol- and quinone-containing vascular disrupting agents have been shown to have enhanced therapeutic activity, relative to vascular disrupting agents that act solely through interaction with tubulin, particularly against solid tumors (see co-pending USSN 10/790,662, U.S. Patent Publication No. 2004-024696, incorporated herein by reference in its entirety).

[0004] In this report, we show that combretastatin compounds that comprise a catechol or quinone moiety induce rapid cell death of non-adherent leukemic cells, at low, non-toxic doses. We also demonstrate that single-agent treatment with a catechol- or quinone-containing combretastatin compound is effective in eradicating both circulating, and vascular-adherent leukemic cells in subcutaneous mouse models of AML, without affecting

normal hematopoiesis. CA1dP-treated mice had significantly prolonged survival and significantly decreased tumor load. Co-administration of an additional chemotherapeutic agent, e.g. AraC, decreases tumor load even further. Therefore, catechol- or quinone-containing combretastatin compounds delivered alone or in combination with chemotherapeutic agents represent a promising novel therapeutic approach to eradicate hematopoietic neoplasms.

[0005] WO2004/078126 and US2006/0035868 report various combretastatin compounds and their use in treating solid tumors.

II. SUMMARY OF THE INVENTION

[0006] The present invention is defined by the claims.

[0007] One aspect of the invention provides a combretastatin compound, or a pharmaceutically acceptable salt thereof, for use in methods of treating a non-solid hematopoietic neoplasm wherein the hematopoietic neoplasm is a myeloid neoplasm, wherein the combretastatin is combretastatin A-1 diphosphate (CA1dP), or a pharmaceutically acceptable salt thereof.

[0008] The invention also contemplates use of a combretastatin capable of forming a reactive oxygen species *in vivo* which is combretastatin A-1 diphosphate (CA1dP) in the preparation of a medicament for use in treating a non-solid hematopoietic neoplasm wherein the hematopoietic neoplasm is a myeloid neoplasm.

[0009] Also described herein are methods of treating a non-solid tumor comprising administering, to a subject suffering from non-solid tumor, a therapeutically effective amount of combretastatin compound or a pharmaceutically acceptable salt thereof, wherein the combretastatin compound comprises a catechol or quinone moiety and is capable of forming a reactive oxygen species *in vivo*.

III. BRIEF DESCRIPTION OF THE DRAWINGS

[0010]

Figure 1 illustrates average tumor volume (\pm SD) as a function of time for subcutaneous HL60 tumors treated with CA4P, CA1dP and/or AraC.

Figures 2 and 3 provide individual tumor volumes for selected experiments summarized in Figure 1.

Figure 4 illustrates the dose response activity of CA1dP (\pm AraC) against leukemia cells.

IV. DETAILED DESCRIPTION

A. Definitions

[0011] 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.

[0012] 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.

[0013] "Catechol" is any group of optionally substituted compounds with aryl functionality and containing at least two OH groups at ortho or para positions on the aryl ring, wherein a conjugated system is formed with at least one C=C bond. The preferred catechol of the present invention is an ortho-benzocatechol. The term "catechol" also encompasses catechols in prodrug form, wherein one or both hydroxyl groups are substituted with a moiety that is metabolically converted to hydroxyl *in vivo*.

[0014] "Quinone" is any group of optionally substituted aromatic polyketone compounds derived from a compound with an aryl moiety. At least two C=O groups are in the ortho or para position on the aryl ring, and form a conjugated system with at least one C=C bond. The preferred quinone of the present invention is an ortho-benzoquinone. Quinones can be synthesized in a number of ways by oxidation of a phenolic precursor such as ortho-catechol. The oxidant reagents used in the reaction can include Jones reagent (Chromate salts), Fremy's salt ((KSO₃)₂NO), and the like. Such oxidation also can occur *in vivo* by virtue of chemical or enzymatic activity. The preferred oxidant is o-iodoxybenzoic acid.

[0015] "Alkyl" refers to monovalent saturated aliphatic hydrocarbyl groups having from 1 to 10 carbon atoms and preferably 1 to 6 carbon atoms. This term includes, by way of example, linear and branched hydrocarbyl groups such as methyl (CH_3-), ethyl (CH_3CH_2-), n-propyl ($\text{CH}_3\text{CH}_2\text{CH}_2-$), isopropyl ($(\text{CH}_3)_2\text{CH}-$), n-butyl ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2-$), isobutyl ($(\text{CH}_3)_2\text{CHCH}_2-$), sec-butyl ($(\text{CH}_3)(\text{CH}_3\text{CH}_2)\text{CH}-$), t-butyl ($(\text{CH}_3)_3\text{C}-$), n-pentyl ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$), and neopentyl ($(\text{CH}_3)_3\text{CCH}_2-$).

[0016] "Alkoxy" refers to the group -O-alkyl, wherein alkyl is as defined herein. Alkoxy includes, by way of example, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, t-butoxy, sec-butoxy, n-pentoxy, and the like.

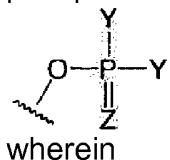
[0017] "Aryl" refers to a monovalent aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (e.g., phenyl) or multiple condensed rings (e.g., naphthyl or anthryl) which condensed rings may or may not be aromatic (e.g., 2-benzoxazolinone, 2H-1,4-benzoxazin-3(4H)-one-7-yl, and the like), provided that the point of attachment is through an atom of the aromatic aryl group. Preferred aryl groups include phenyl and naphthyl.

[0018] "Halo" or "halogen" refers to fluoro, chloro, bromo, and iodo and is preferably fluoro or chloro.

[0019] "Hydroxy" or "hydroxyl" refers to the group -OH.

[0020] As used herein, the term "prodrug" refers to a precursor form of the drug which is metabolically converted *in vivo* to produce the active drug. Thus, for example, combretastatin phosphate prodrug salts administered to an animal in accordance with the present invention undergo metabolic activation and regenerate combretastatin A-1 *in vivo*, e.g., following dissociation and exposure to endogenous non-specific phosphatases in the body, the drug which is metabolically converted *in vivo* to produce the active drug. Prodrugs include the phosphate, phosphoramidate, or amino acid acyl groups as defined herein. The phosphate ester salt moiety may also include $(-\text{OP}(\text{O})(\text{O-alkyl})_2$ or $(-\text{OP}(\text{O})(\text{O-}\text{NH}_4^+)_2$). A prodrug can comprise a substitution of a phenolic moiety or amine moiety of the active drug with a phosphate, phosphoramidate, or amino acid acyl group. A wide variety of methods for the preparation of prodrugs are known to those skilled in the art (see, for example, Pettit and Lippert, *Anti-Cancer Drug Design*, (2000), 15, 203-216).

[0021] Catechol prodrugs are precursor forms of catechols that are metabolically converted *in vivo* to produce corresponding catechols. The catechol prodrug can comprise at least one phosphate replacing a hydroxyl moiety of the catechol. typically, the phosphate moiety has the general formula:



each Y independently is OR or NHR, S⁻, NH⁻ or O⁻, wherein each R independently is selected from hydrogen, C₁₋₆ alkyl, a mono or divalent metal cation, an ammonium cation;

and
Z is O or S.

[0022] "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.

[0023] 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).

[0024] 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.

[0025] "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.

[0026] "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.

[0027] 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.

[0028] 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. WO02/22626 or WO00/48606 and US Patent 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 US Patent No. 7,018,987, which is incorporated by reference herein.

B. Methods of Treating Hematopoietic Neoplasms

[0029] 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 the catechol compound combretastatin A-1 diphosphate (CA1dP) inhibit leukemic cell proliferation *in vitro* and induce mitotic arrest and cell death. In addition, CA1dP rapidly increases intracellular reactive oxygen species (ROS), and antioxidant treatment provides partial protection from cell death. As such, combretastatin compounds comprising a catechol or quinone moiety,

such as CA1dP, provide for an effective means to treat refractory organ-infiltrating leukemias.

[0030] Accordingly, described herein is a method of treating a hematopoietic neoplasm, the method comprising administering, to a mammal suffering from a hematopoietic neoplasm, a therapeutically effective amount of a combretastatin compound comprising a catechol or quinone moiety. Preferably the combretastatin compound is combretastatin A1, a combretastatin A-1 prodrug (such as combretastatin A-1 diphosphate), or a pharmaceutically acceptable salt thereof.

[0031] Derived from the South African tree *Combretum caffrum*, combretastatins such as combretastatin A-4 (CA-4) were initially identified in the 1980's as a potent inhibitors of tubulin polymerization. CA-4, and other combretastatins (e.g. CA-1) have been shown to bind 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. CA4P and CA1P, respective phosphate prodrugs of CA-4 and CA-1, were subsequently developed to combat problems with aqueous insolubility. Surprisingly, CA1P 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 shut-down 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 CA1P than blood flow to tumors, although blood flow to some organs, such as spleen, skin, skeletal muscle and brain, can be transiently inhibited.

[0032] As used herein, the term "combretastatin" or "combretastatin compound" denotes at least one of the combretastatin family of compounds, derivatives or analogs thereof, their prodrugs (preferably phosphate prodrugs) and derivatives thereof, and salts of these compounds. Combretastatins include those anti-cancer compounds isolated from the South African tree *Combretum caffrum*, including without limitation, Combretastatins A-1, A-2, A-3, A-4, B-1, B-2, B-3, B-4, D-1, and D-2, and various prodrugs thereof, exemplified by Combretastatin A-4 phosphate (CA4P) compounds, Combretastatin A-1 diphosphate (CA1dP) compounds and salts thereof (see for example Pettit et al, Can. J. Chem., (1982); Pettit et al., J. Org. Chem., 1985; Pettit et al., J. Nat. Prod., 1987; Lin et al., Biochemistry, (1989); Pettit et al., J. Med. Chem., 1995; Pettit et al., Anticancer Drug Design, (2000); Pettit et al., Anticancer Drug Design, 16(4-5): 185-93 (2001)).

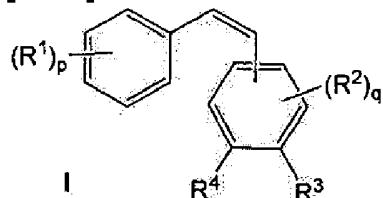
[0033] Combretastatin and combretastatin salts contemplated for use in the methods of the invention are described in WO 99/35150; WO 01/81355; WO 02/022626; US Patent Nos. 4,996,237; 5,409,953; 5,561,122; 5,569,786; 6,538,038; 6,670,344; 6,855,702; 7,018,987; 7,078,552; and 7,279,466. Derivatives or analogs of combretastatins also are described in Singh et al., J. Org. Chem., 1989; Cushman et al, J. Med. Chem., 1991; Getahun et al, J. Med. Chem., 1992; Andres et al, Bioorg. Med. Chem. Lett., 1993; Mannila, et al., Liebigs. Ann. Chem., 1993; Shirai et al., Bioorg. Med. Chem. Lett., 1994; Medarde et al., Bioorg. Med. Chem. Lett., 1995; Wood et al, Br. J. Cancer, 1995; Bedford

et al., *Bioorg. Med. Chem. Lett.*, 1996; Dorr et al., *Invest. New Drugs*, 1996; Jonnalagadda et al., *Bioorg. Med. Chem. Lett.*, 1996; Shirai et al., *Heterocycles*, 1997; Aleksandrzak, et al., *Anticancer Drugs*, 1998; Chen et al., *Biochem. Pharmacol.*, 1998; Ducki et al., *Bioorg. Med. Chem. Lett.*, 1998; Hatanaka et al., *Bioorg. Med. Chem. Lett.*, 1998; Medarde et al., *Eur. J. Med. Chem.*, 1998; Medina et al., *Bioorg. Med. Chem. Lett.*, 1998; Ohsumi et al., *Bioorg. Med. Chem. Lett.*, 1998; Ohsumi et al., *J. Med. Chem.*, 1998; Pettit, et al., *J. Med. Chem.*, 1998; Shirai et al., *Bioorg. Med. Chem. Lett.*, 1998; Banwell et al., *Aust. J. Chem.*, 1999; Medarde et al., *Bioorg. Med. Chem. Lett.*, 1999; Shan et al., *PNAS*, 1999; Combeau et al., *Mol. Pharmacol.*, 2000; Pettit et al., *J. Med. Chem.*, 2000; Pinney et al., *Bioorg. Med. Chem. Lett.*, 2000; Flynn et al., *Bioorg. Med. Chem. Lett.*, 2001; Gwaltney et al., *Bioorg. Med. Chem. Lett.*, 2001; Lawrence et al., 2001; Nguyen-Hai et al., *Bioorg. Med. Chem. Lett.*, 2001; Xia et al., *J. Med. Chem.*, 2001; Tahir et al., *Cancer Res.*, 2001; Wu-Wong et al., *Cancer Res.*, 2001; Janik et al., *Bioorg. Med. Chem. Lett.*, 2002; Kim et al., *Bioorg. Med. Chem. Lett.*, 2002; Li et al., *Bioorg. Med. Chem. Lett.*, 2002; Nam et al., *Bioorg. Med. Chem. Lett.*, 2002; Wang et al., *J. Med. Chem.* 2002; Hsieh et al., *Bioorg. Med. Chem. Lett.*, 2003; Hadimani et al., *Bioorg. Med. Chem. Lett.*, 2003; Mu et al., *J. Med. Chem.*, 2003; Nam et al., *Curr. Med. Chem.*, 2003; Pettit et al., *J. Med. Chem.*, 2003; Gaukroger et al., *Org. Biomol. Chem.* 2003; Bailly et al., *J. Med. Chem.* 2003; Sun et al., *Anticancer Res.* 2004; Sun et al., *Bioorg. Med. Chem. Lett.* 2004; Liou et al., *J. Med. Chem.* 2004; Perez-Melero et al., *Bioorg. Med. Chem. Lett.* 2004; Liou et al., *J. Med. Chem.* 2004; Mamane et al., *Chemistry*, 2004; De Martini et al., *J. Med. Chem.* 2004; Ducki et al., *J. Med. Chem.* 2005; Maya et al., *J. Med. Chem.* 2005; Medarde et al., *J. Enzyme Inhib. Med. Chem.* 2004; Simoni et al., *J. Med. Chem.* 2005; Sanchez et al., *Bioorg. Med. Chem.* 2005; Vongvanich et al., *Planta Med.* 2005; Tron et al., *J. Med. Chem.* 2005; Borrel et al., *Bioorg. Med. Chem.* 2005; Hsieh et al., *Curr. Pharm. Des.* 2005; Lawrence et al., *Curr. Pharm. Des.* 2005; Hadfield et al., *Eur. J. Med. Chem.* 2005; Pettit et al., *J. Med. Chem.* 2005; Coggioloa et al., *Bioorg. Med. Chem. Lett.* 2005; Kaffy et al., *Org. Biomol. Chem.* 2005; Mateo et al., *J. Org. Chem.* 2005; LeBlanc et al., *Bioorg. Med. Chem.* 2005; Srivastava et al., *Bioorg. Med. Chem.* 2005; Nguyen et al., *J. Med. Chem.* 2005; Kong et al., *Chem. Biol.* 2005; Li et al., *Bioorg. Med. Chem. Lett.* 2005; Pettit et al., *J. Nat. Prod.* 2005; Nicholson et al., *Anticancer Drugs*, 2006; Monk et al., *Bioorg. Med. Chem.* 2006; De Martino et al., *J. Med. Chem.* 2006; Peifer et al., *J. Med. Chem.* 2006; Kaffy et al., *Bioorg. Med. Chem.* 2006; Banwell et al., *Bioorg. Med. Chem.* 2006; Dupeyre et al., *Bioorg. Med. Chem.* 2006; Simoni et al., *J. Med. Chem.* 2006; Tron et al., *J. Med. Chem.* 2006; Romagnoli et al., *J. Med. Chem.* 2006; Pandit et al., *Bioorg. Med. Chem.* 2006; Nakamura et al., *ChemMedChem*, 2006; Pirali et al., *J. Med. Chem.* 2006; Bellina et al., *Bioorg. Med. Chem. Lett.* 2006; Hu et al., *J. Med. Chem.* 2006; Chang et al., *J. Med. Chem.* 2006; Thomson et al., *Mol. Cancer Ther.* 2006; Fortin et al., *Bioorg. Med. Chem. Lett.*, 2007; Duan et al., *J. Med. Chem.*, 2007; Zhang et al., *J. Med. Chem.* 2007; Wu et al., *Bioorg. Med. Chem. Lett.* 2007; Sun et al., *Bioorg. Med. Chem. Lett.* 2007, WO 06/138427; WO 036743; WO 05/007635, WO 03/040077, WO 03/035008, WO 02/50007, WO 02/14329; WO 01/12579, WO 01/09103, WO 01/81288, WO 01/84929, WO 00/48590, WO 00/73264, WO 00/06556, WO 00/35865, WO 99/34788, WO 99/48495, WO 92/16486, US Patent Nos. 7,125,906; 7,105,695; 7,105,501; 7,087,627; 7,030,123; 7,078,552; 7,030,123; 7,018,987; 6,992,106; 6,919,324; 6,846,192, 6,855,702; 6,849,656; 6,794,384; 6,787,672, 6,777,578, 6,723,858, 6,720,323, 6,433,012, 6,423,753, 6,201,001, 6,150,407, 6,169,104, 5,731,353, 5,674,906, 5,430,062, 5,525,632, 4,996,237 and 4,940,726.

[0034] As described herein, the combretastatin comprises a quinone or catechol,

preferable an orthoquinone or ortho-catechol. Oxidation of an ortho-catechol, such as CA1, to an orthoquinone can result in oxidative damage to neoplastic cells via redox cycling. This is a process in which the quinone is reduced to a radical (ie. semiquinone), which in turn reduces oxygen to superoxide radicals with the quinone being reformed or cycled. The generation of a quinone derivative from CA1 has been demonstrated *in vivo* and the quinone was found to react rapidly with the reducing agents glutathione and ascorbate. In addition, rapid consumption of oxygen in the presence of ascorbate confirmed the formation of CA1 quinone. Furthermore, redox-cycling, confirming the formation of CA1 semiquinone, was observed with CA1.

[0035] Described herein is a combretastatin compound of the Formula I:



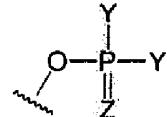
or a pharmaceutically acceptable salt thereof, wherein each R¹ independently is selected from C₁₋₆ alkoxy, halogen and halo-substituted C₁₋₆ alkyl;

each R² independently is selected from C₁₋₆ alkoxy, halogen and halo-substituted C₁₋₆ alkyl;

p is 1, 2, 3, or 4;

q is 0, 1 or 2;

R³ and R⁴ each independently is selected from hydroxyl or

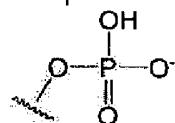


wherein

each Y independently is OR⁵ or NHR⁵, or O⁻, wherein each R⁵ independently is selected from hydrogen and C₁₋₆ alkyl;

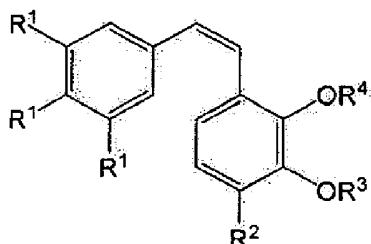
Z is O or S.

[0036] Also described herein, the compound is a pharmaceutically acceptable salt of the compound of Formula I, wherein each of R³ and R⁴ is a phosphate:



wherein the cation of the salt can be either a divalent cation or two monovalent cations. Preferably, the divalent cation is a divalent metal cation. In some implementations, the monovalent cation is an alkali metal, an aliphatic amine or an ammonium. In other implementations, the monovalent cation 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 hydrazamine.

[0037] Also described herein, Formula I is represented by a compound of Formula II:

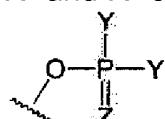


wherein

each R¹ independently is selected from C₁₋₆ alkoxy, halogen and halo-substituted C₁₋₆ alkyl;

R² is selected from C₁₋₆ alkoxy, halogen and halo-substituted C₁₋₆ alkyl;

R³ and R⁴ each independently is selected from hydroxyl or

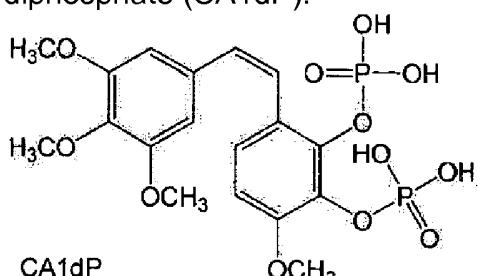


wherein

each Y independently is OR⁵ or NHR⁵, or O⁻, wherein each R⁵ independently is selected from hydrogen and C₁₋₆ alkyl;

Z is O or S.

[0038] According to the present invention, the compound is combretastatin A-1 diphosphate (CA1dP):



or a pharmaceutically acceptable salt thereof.

[0039] The method described herein 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, CA1dP and 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 CA1dP and a Ara-C at the same time) and time varied administration (administration of CA1dP at a time different from that of the Ara-C), as long as both the combretastatin A-1 diphosphate, 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.

[0040] 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, triethylenephosphoramide, triethylenethiophosphoramide 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, chlophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gamma1l and calicheamicin omegal1 (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 antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, 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, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine (Ara-C), dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitostanol, 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; demecoicine; diaziquone; el fornithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine, and ansamitocins; mitoguazone; mitoxantrone; moidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, e.g., paclitaxel (TAXOL®), albumin-engineered nanoparticle formulation

of paclitaxel (ABRAXANE™), and doxetaxel (TAXOTERE®); chlorambucil; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovorin; 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 leucovorin.

[0041] 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 is a hematopoietic neoplasm, which is aberrant growth of cells of the hematopoietic system. Hematopoietic malignancies can have their 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.

[0042] 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 neoplastic cells 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 combretastatins and combretastatin compounds comprising a catechol or quinone moiety, can selectively induce apoptosis of circulating and vascular-bound leukemic cells, leading to cell death. This induction of apoptosis occurs by a caspase-dependent mechanism as well as ROS-mediated mitochondrial damage. Thus, combretastatin compounds comprising a catechol or quinone moiety are effective in treating hematopoietic neoplasms, as demonstrated by their ability to target hematopoietic neoplasms *in vitro* and *in vivo* and to eradicate circulating, marrow- and organ-resident vascular-adherent hematopoietic neoplasms.

[0043] Also described herein, 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 the present invention, the non-solid 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)(q34;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 non-solid myeloid neoplasms can be treated with the combretastatin compounds of the invention.

[0044] In some implementations, the combretastatin compounds of the invention 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.

[0045] One aspect of the invention is a pharmaceutical composition useful for treating a non-solid hematopoietic neoplasm in a mammal wherein the hematopoietic neoplasm is a myeloid neoplasm, which composition comprises combretastatin A-1 diphosphate (CA1dP) 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 are 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.

[0046] 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.

[0047] A unit dose will contain a therapeutically effective amount sufficient to treat a non-solid hematopoietic neoplasm wherein the hematopoietic neoplasm is a myeloid neoplasm in a subject and may contain from about 1.0 to 1000 mg of compound, for example about 50 to 500 mg.

[0048] The combretastatin compound 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.

[0049] 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.

[0050] 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.

[0051] 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.

[0052] 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.

[0053] 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, thimerosal, or the like. It may also be preferable to include agents that alter the tonicity such as sugars or salts.

[0054] The combretastatin compound also can be administered orally in a suitable formulation as an ingestible tablet, a buccal tablet, capsule, caplet, elixir, suspension, syrup, trouche, wafer, lozenge, and the like. Generally, the most straightforward formulation is a tablet or capsule (individually or collectively designated as an "oral dosage unit"). Suitable formulations are prepared in accordance with a standard formulating techniques available that match the characteristics of the compound to the excipients available for formulating an appropriate composition. A tablet or capsule will preferably contain about 50 to about 500 mg of such a combretastatin compound.

[0055] The form may deliver a compound rapidly or may be a sustained-release preparation. The compound may be enclosed in a hard or soft capsule, may be compressed into tablets, or may be incorporated with beverages, food or otherwise into the diet. The percentage of the final composition and the preparations may, of course, be varied and may conveniently range between 1 and 90% of the weight of the final form, e.g., tablet. The amount in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions according to the current invention are prepared so that an oral dosage unit form contains between about 5.0 to about 50% by weight (%w) in dosage units weighing between 5 and 1000 mg.

[0056] The suitable formulation of an oral dosage unit may also contain: a binder, such as gum tragacanth, acacia, corn starch, gelatin; sweetening agents such as lactose or sucrose; disintegrating agents such as corn starch, alginic acid and the like; a lubricant such as magnesium stearate; or flavoring such a peppermint, oil of wintergreen or the like. Various other materials may be present as coating or to otherwise modify the physical form of the oral dosage unit. The oral dosage unit may be coated with shellac, a sugar or both. Syrup or elixir may contain the compound, sucrose as a sweetening agent, methyl and propylparabens as a preservative, a dye and flavoring. Any material utilized should be pharmaceutically-acceptable and substantially non-toxic. Details of the types of excipients useful may be found in the nineteenth edition of "Remington: The Science and Practice of Pharmacy," Mack Printing Company, Easton, Pa. See particularly chapters 91-93 for a fuller discussion.

[0057] Another aspect of this invention is a therapeutically effective amount of combretastatin A-1 diphosphate (CA1dP) or a pharmaceutically acceptable salt thereof for use in treating a non-solid hematopoietic neoplasm in a warm-blooded animal wherein the hematopoietic neoplasm is a myeloid neoplasm, . A combretastatin compound useful in this invention is administered to an appropriate subject in need of these agents 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.

[0058] 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/m² 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 m² body surface area of a compound of the invention, for instance from 50 to 500 mg/m².

[0059] Another important feature of the compound for use in accordance with 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.

V. EXAMPLES

A. EXAMPLE 1: Subcutaneous *in vivo* leukemia model

[0060] HL60 (1×10^7 cells in 0.1 ml) were injected subcutaneously into the dorsa of 4-6 week old athymic nu/nu mice (Southern Research Institute). When palpable tumors were evident (average tumor volume 100mm^3 ; i.e. approximately 12 days after inoculation), 6 experimental groups of mice were randomized, each with 10 animals. Treatment was initiated according to the following regimens:

Group	Animals	Compound	Dose	Treatment Schedule
1	10	Control (PBS)	0	Q4D x 2/2 wks (SD) - Q3Hx3 ()
2	10	CA1dP	75 mg/kg/inj	Q7Dx2 (SD+1)
3	10	CA4P	75 mg/kg/inj	Q7Dx2 (SD+1)
4	10	Ara-C	20 mg/kg/inj	Q4Dx2/2 wks (SD) - Q3Hx3 ()
5	10	CA1dP	75 mg/kg/inj	Q7Dx2 (SD+1)
		Ara-C	20 mg/kg/inj	Q4Dx2/2 wks (SD) - Q3Hx3 ()
6	10	CA4P	75 mg/kg/inj	Q7Dx2 (SD+1)
		Ara-C	20 mg/kg/inj	Q4Dx2/2 wks (SD) - Q3Hx3 ()

[0061] Tumor volume was measured three times a week until the endpoint was reached (tumor volume $>3000\text{ mm}^3$), or for 90 days post-treatment. Treatment with Ara-C alone showed little improvement over treatment with phosphate buffered saline (control). Treatment with CA4P as a single agent showed delayed tumor growth and extended life span as compared to controls (See Figure 1). Treatment with an orthoquinone prodrug, CA1dP, demonstrated substantial decreases in tumor volume and five complete tumor regressions in a population of ten treated mice (Figure 2). Addition of Ara-C to CA1dP further decreased tumor volumes and led to six complete regressions out of ten treated mice (Figure 3).

A. EXAMPLE 2: Dose Response Activity

[0062] HL60 (1×10^7 cells in 0.1 ml) were injected subcutaneously into the dorsa of 4-6 week old athymic nu/nu mice (Southern Research Institute). When palpable tumors were evident (average tumor volume 100mm^3 ; i.e. approximately 12 days after inoculation), ten

experimental groups of mice were randomized, each with 10 animals. Treatment was initiated according to the following regimens:

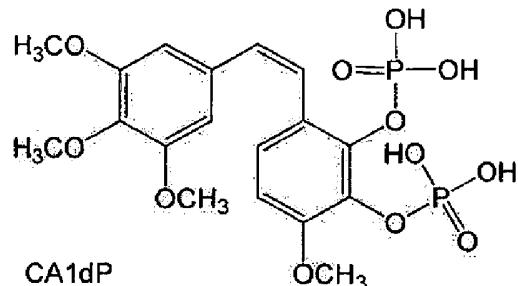
Group	Compound	Dose	Treatment Schedule
1	Control	0 mg/kg/inj	
2	CA1dP	75 mg/kg/inj	day 3 and 10
3	CA1dP	25 mg/kg/inj	day 3 and 10
4	CA1dP	10 mg/kg/inj	3 and 10
5	CA1dP	2.5 mg/kg/inj	day 3 and 10
6	Ara-C	20 mg/kg/inj	tid day 1, 4, 8 and 12
7	Ara-C +CA1dP	20 mg/kg/inj	tid day 1, 4, 8 and 12
		75 mg/kg/inj	day 3 and 10
8	Ara-C +CA1dP	20 mg/kg/inj	tid day 1, 4, 8 and 12
		25 mg/kg/inj	day 3 and 10
9	Ara-C +CA1dP	20 mg/kg/inj	tid day 1, 4, 8 and 12
		10 mg/kg/inj	day 3 and 10
10	Ara-C +CA1dP	20 mg/kg/inj	tid day 1, 4, 8 and 12
		2.5 mg/kg/inj	day 3 and 10

[0063] Tumor volume was measured three times per week. The decrease in tumor volume showed a clear dose response relationship relative to the dose of CA1dP administered (See Figure 4), administration of doses of CA1dP above 2.5 mg/kg showed significant anti-tumor activity. Although the number of complete responses was not significantly altered by the combination with araC, tumors showed longer growth inhibition following treatment with CA1dP and araC as compared to tumors treated with CA1dP alone.

[0064] Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in the art are intended to be within the scope of the following claims.

Patentkrav

1. Combretastatin-forbindelse, eller et farmaceutisk acceptabelt salt deraf, til
anvendelse ved behandling af en ikke-fast hæmatopoietisk neoplasme, hvor
den hæmatopoietiske neoplasme er en myeloid neoplasme, og hvor combre-
tastatin-forbindelsen er combretastatin A-1-diphosphat (CA1dP):



eller et farmaceutisk acceptabelt salt deraf.

10 **2.** Forbindelse til anvendelse ifølge krav 1, hvor forbindelsen er et farmaceutisk acceptabelt salt, hvor saltets kation enten kan være en divalent kation eller to monovalente kationer.

15 **3.** Forbindelse til anvendelse ifølge krav 2, hvor den divalente kation er en divalent metalkation.

20 **4.** Forbindelse til anvendelse ifølge krav 2, hvor den monovalente kation er et alkalinmetal, en alifatisk amin eller et ammonium.

25 **5.** Forbindelse til anvendelse ifølge krav 2, hvor den monovalente kation er valgt fra gruppen bestående af natrium, TRIS, histidin, ethanolamin, diethanolamin, ethylendiamin, diethylamin, triethanolamin, glucamin, N-methylglucamin, ethylendiamin, 2-(4-imidazolyl)-ethylamin, cholin og hydramin.

30 **6.** Forbindelse til anvendelse ifølge krav 1, hvor den myeloide neoplasme er akut myeloid leukæmi (AML).

35 **7.** Forbindelse til anvendelse ifølge krav 1, hvor den myeloide neoplasme er refraktær organ-infiltrerende leukæmi.

- 8.** Forbindelse til anvendelse ifølge krav 1, hvor det farmaceutisk acceptable salt er et kaliumsalt af combretastatin A-1-diphosphat.
- 9.** Forbindelse til anvendelse ifølge krav 1, endvidere omfattende et kemoterapeutisk middel.
- 10.** Forbindelse til anvendelse ifølge krav 9, hvor det kemoterapeutiske middel er Ara-C, etoposid, thioguanin eller cyclophosphamid.

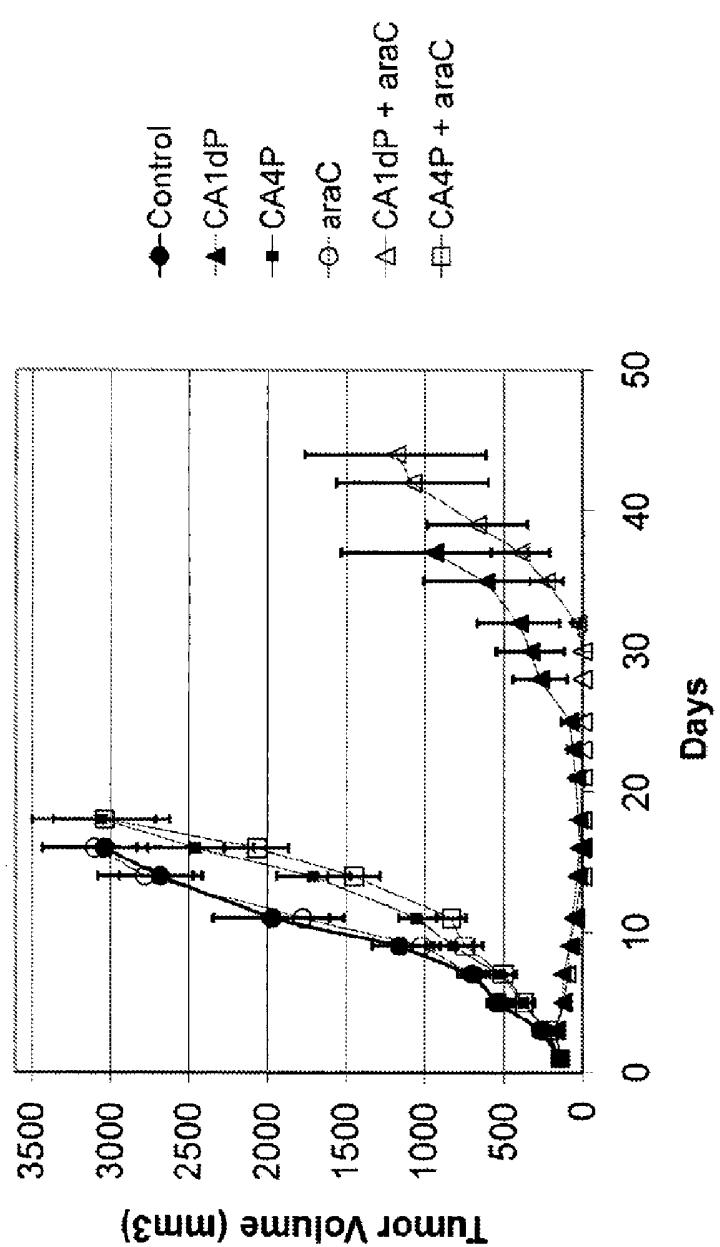


FIGURE 1

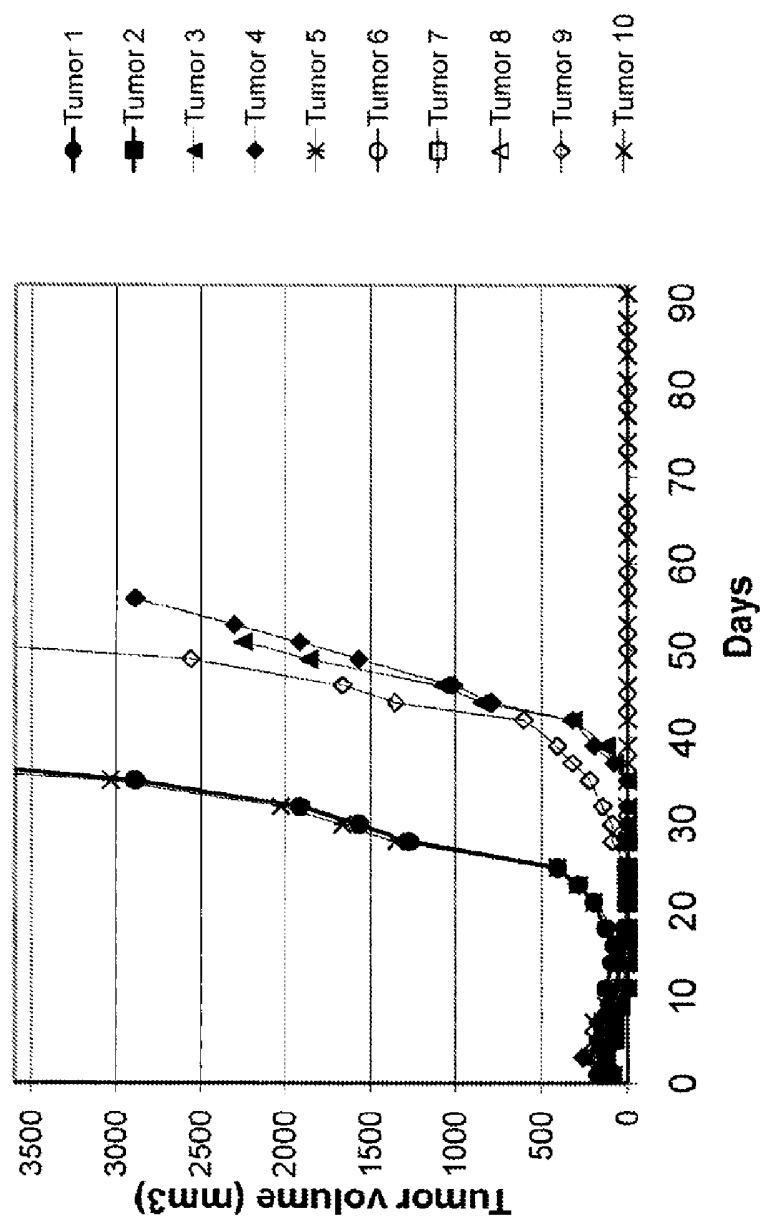


FIGURE 2

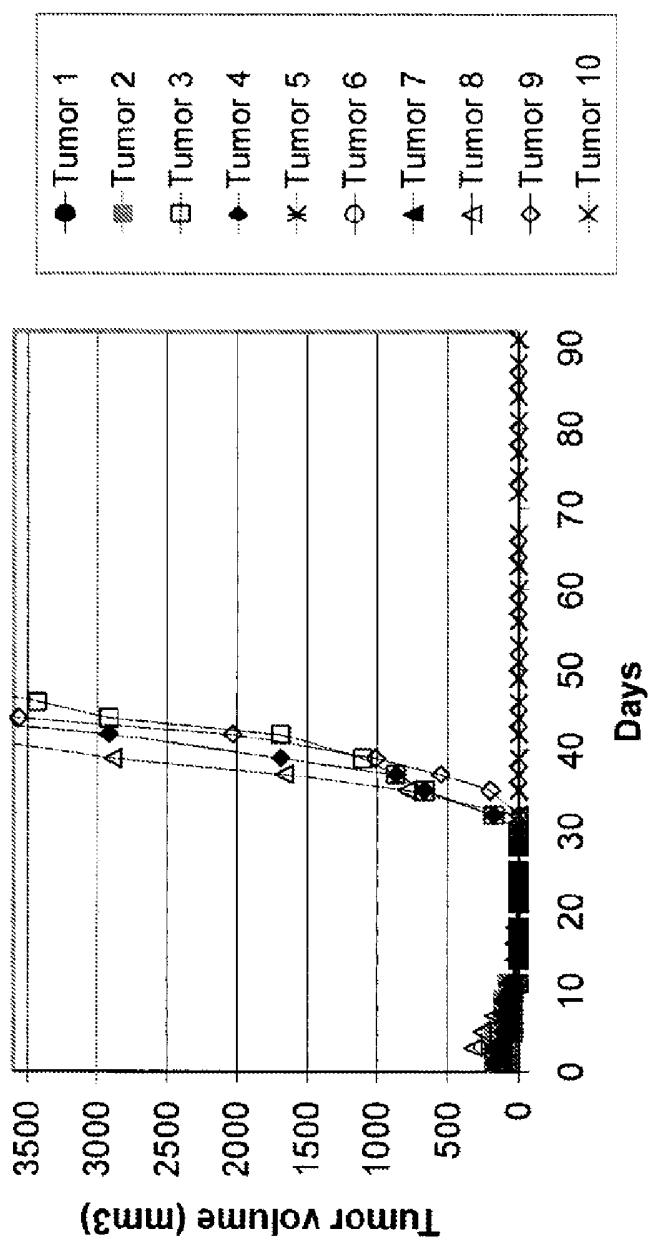


FIGURE 3

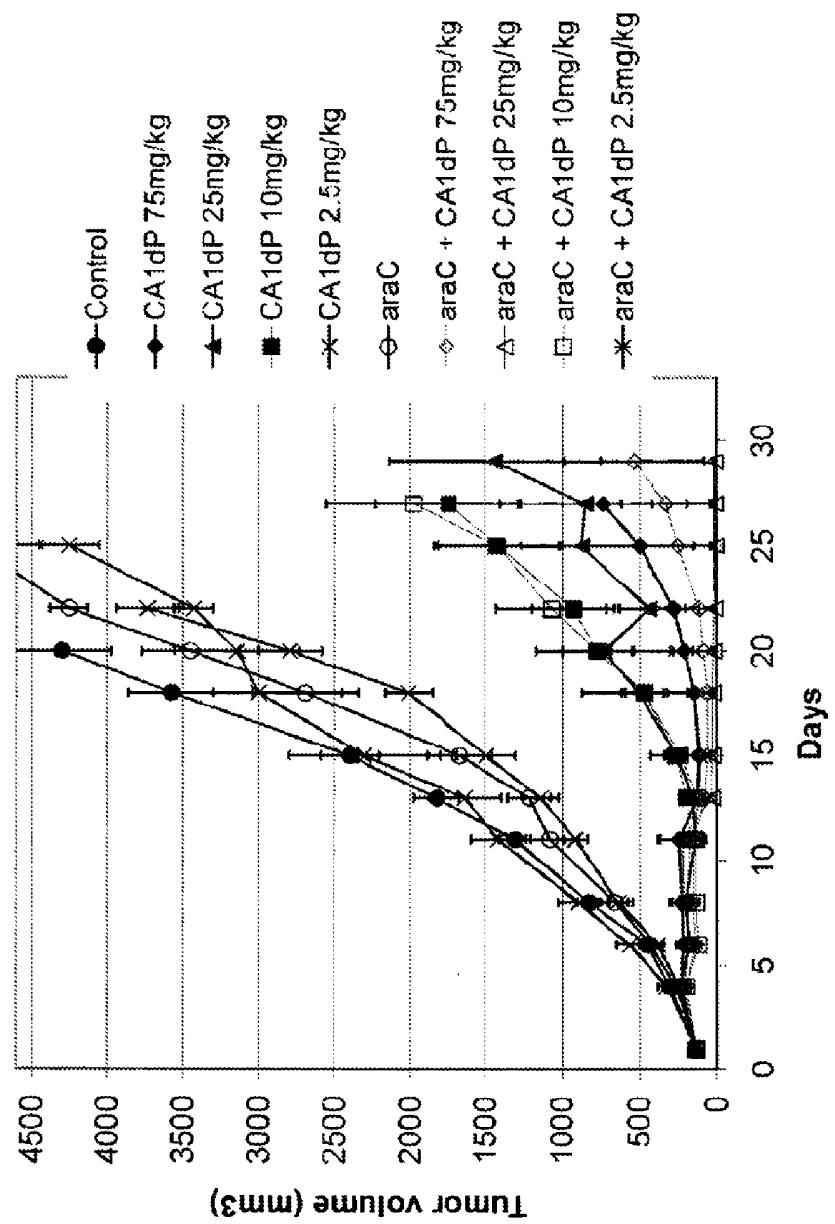


FIGURE 4