PATENT ASSIGNMENT COVER SHEET

Electronic Version v1.1
Stylesheet Version v1.2

EPAS ID: PAT5304359

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	SECURITY INTEREST

CONVEYING PARTY DATA

Name	Execution Date
BIOSUCCESS BIOTECH CO., LTD.	12/31/2018

RECEIVING PARTY DATA

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PROPERTY NUMBERS Total: 1

Property Type	Number
Patent Number:	9132113

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ATTORNEY DOCKET NUMBER:	A207062
NAME OF SUBMITTER:	NONA MCKOY, SECRETARY
SIGNATURE:	/Nona McKoy/
DATE SIGNED:	12/31/2018

Total Attachments: 68
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INTELLECTUAL PROPERTY SECURITY AGREEMENT

This Intellectual Property Security Agreement ("Agreement"), dated as of the date signed by both parties, is made by Biosuccess Biotech Co., Ltd. (the "Debtor"), with its chief executive office and mailing address at c/o Biosuccess Biotech Co. Ltd., Suite 200, 2570 North First Street, San Jose, CA 95121, USA in favor of Sughrue Mion, PLLC, 2100 Pennsylvania Ave., NW, Washington DC 20037 in its capacity as "Secured Party" under the Agreement referenced herein below.

WITNESSETH:

WHEREAS, Secured Party has done substantial legal work on Debtor's intellectual property portfolio, including patents and patent applications, for which Secured Party has not been paid;

WHEREAS, Debtor agrees that the amounts indicated on Secured Party's monthly statements of account, and related correspondence, are overdue and owed ("the Obligations");

WHEREAS, pursuant to the Agreement herein, Debtor has granted to Secured Party, for its benefit, security interests in and to and Liens on certain of Debtor's Intellectual Property and specifically including all of Debtor's registered United States patents and all of Debtor's filed United States applications, all whether now owned or hereafter created, arising and/or acquired (collectively, the "US Registered Intellectual Property"); and

WHEREAS, Debtor has agreed to execute and deliver this Agreement and to have a copy of this Agreement filed with the United States Patent and Trademark Office and any appropriate state or local government offices, in order to perfect and/or protect all of Secured Party's Liens in the US Registered Intellectual Property.

NOW THEREFORE, in consideration of the premises and mutual covenants and agreements set forth herein, and for other good and valuable consideration, the receipt and sufficiency of which are hereby acknowledged by each party hereto, and with the intention of being legally bound hereby, the Debtor and the Secured Party agree as follows:

SECTION 1. Grant of Security Interest. The Debtor hereby grants to the Secured Party a lien on and continuing security interest in all of such Debtor's right, title, and interest in and to the following:

(a) all granted patents and pending patent applications in the United States (and all patents that issue therefrom), including all industrial designs, industrial models, utility models, certificates of invention and other indices of invention ownership, and all reissues, reexaminations, extensions, renewals, substitutes, divisions and continuations (including continuations-in-part and continuing prosecution applications) thereof, all rights to make applications for issuance and recordations, for the full term thereof, now existing or hereafter applied for, issued, or acquired ("Patents"), and

(b) any agreement, whether written or oral, providing for the grant of any right under any of the foregoing Patents ("IP Licenses"), including, without limitation, those items listed on Schedule A hereto (the foregoing collectively, the "IP Collateral"), as collateral security for the prompt and complete payment and performance when due (whether at the stated maturity, by acceleration or otherwise) of the Debtor's Obligations ("Obligations"), provided that the security interest created hereby does not constitute an assignment, shall not include any license or agreement to the extent that, and only during the period in which, a grant of security therein would violate or create a right of termination in favor of any other party thereto (other than any Debtor) to the extent such is not rendered unenforceable pursuant to applicable provisions of the UCC or other applicable law, and shall not include any of the foregoing to the extent that, and only during the period in which, a grant of security interest therein would void, invalidate, cancel, or impair the enforceability of such intellectual property rights.

SECTION 2. Security for Obligations. The grant of a security interest in the Patents and IP Collateral herein secures the payment of all of the Obligations.

SECTION 3. Recordation. The Debtor authorizes and requests that the United States Patent and Trademark Office, or other governmental agency record this Agreement.

SECTION 4. Debtor Remains Liable. Debtor hereby agrees that, anything herein to the contrary notwithstanding, Debtor shall retain full and complete responsibility for the prosecution, defense, enforcement or any other necessary or desirable actions in connection with their Intellectual Property, subject to this security interest hereunder. Secured Party is not required to continue prosecution of any patent applications in the U.S. or abroad, nor to maintain any granted patents through annuities or the like in the U.S. or abroad.

Section 5. Agreement to Deliver Supplements. Debtor hereby covenants and agrees that promptly upon the acquisition by Debtor of any new US Registered Intellectual Property Collateral (including any registration or issuance of any United States patent arising out of any filed United States patent application listed on Schedule A hereto or the Schedule to any other Supplement delivered to Secured Party in accordance with this paragraph), Debtor shall, deliver to Secured Party a duly executed Supplement to this Agreement listing all such newly acquired US Registered Intellectual Property, pursuant to which Debtor shall reconfirm the grant of a security interest in such newly acquired US Registered Intellectual Property Collateral to Secured Party, for its benefit and for the ratable benefit of Secured Party, to secure the Obligations. Each such Supplement is intended by the parties to be filed, and Debtor hereby authorizes Secured Party to file and record a copy of each such Supplement, with the United States Patent and Trademark Office and/or other local or state government offices, as applicable. Regardless of whether any Supplement is delivered by Debtor, and without limiting the generality of the provisions of Section 1 hereof above, Debtor hereby confirms and agrees that any and all such after-acquired US Registered Intellectual Property Collateral, and all IP Collateral relating thereto, shall immediately and automatically upon Debtor's acquisition of any right, title and interest therein become part of the IP Collateral hereunder. In the event that Debtor acquires any such new US Registered Intellectual Property but Debtor fails for whatever reason to promptly deliver a Supplement pursuant to this Section 5, Debtor hereby authorizes Secured Party, acting under its Power of Attorney

granted pursuant to Section 6 below, at any time thereafter and until the Termination, to execute in the name of Debtor an applicable Supplement with respect to such newly acquired US Registered Intellectual Property, arising and/or acquired and to file the same with the United States Patent and Trademark Office and any other applicable state or local government office.

Section 6. Power of Attorney. Without limiting the generality of any power of attorney granted to Secured Party under any Other Document, Debtor hereby authorizes Secured Party, its successors and assigns, and any officer, employee, attorney or agent thereof, as Debtor's true and lawful attorney-in-fact, with the power to execute and endorse on behalf of and in the name of Debtor any Supplement to this Agreement or other security agreement or similar document or instrument which Secured Party may deem necessary or desirable in order to create, protect, perfect or enforce the security interest in the Patents and/or IP Collateral provided for herein and in each case to file or record any such Supplement or other security agreement or similar document or instrument with the United States Patent and Trademark Office and any other applicable state or local government office, in the name of and on behalf of Debtor, and after the occurrence and during the continuance of an event of default, to execute and endorse on behalf of and in the name of Debtor any assignment, bill of sale or similar document or instrument which Secured Party may deem necessary or desirable in order for Secured Party to assign, pledge, convey or otherwise sell, transfer title in or dispose of the IP Collateral, and in each case to file or record with the United States Patent and Trademark Office and/or state or local government office, as applicable, in the name of and on behalf of Grantor any such assignment or bill of sale or other document executed by Secured Party, its successors and assigns, and any officer, employee, attorney or Secured Party thereof under this power of attorney. Debtor hereby unconditionally ratifies all that any person authorized under this power of attorney shall lawfully do or cause to be done by virtue hereof and in accordance with the terms hereof. This power of attorney is coupled with an interest and cannot be revoked until the Termination.

SECTION 7. Counterparts. This Agreement may be executed in counterparts, each of which will be deemed an original, but all of which together constitute one and the same original.

SECTION 8. Governing Law. This IP Security Agreement shall be governed by, and construed in accordance with, the laws of the District of Columbia. If any part of this Agreement is contrary to, prohibited by, or deemed invalid under Applicable Laws or regulations, such provision shall be inapplicable and deemed omitted to the extent so contrary, prohibited or invalid, but the remainder hereof shall not be invalidated thereby and shall be given effect so far as possible. This Agreement shall be binding upon and inure to the benefit of Debtor and Secured Party, and their respective successors and assigns, except that Debtor may not assign or transfer any of its rights or obligations under this Agreement without the prior written consent of Secured Party.

[SIGNATURE PAGES FOLLOW]

IN WITNESS WHEREOF, the parties hereto have caused this Intellectual Property Security Agreement to be duly executed and delivered by their respective officers thereunto duly authorized as of the Effective Date of December 31, 2018.

DEBTOR:

BIOSUCCESS BIOTECH CO., LTD.

By: _____
Name: Fred Wu
Title: CEO

SECURED PARTY:

SUGHRUE MION, PLLC

By: _____
Name: John F. Rabena
Title: Managing Partner

Schedule A
To Intellectual Property Security Agreement

Debtor's U.S. Issued Patents

Title	Patent No.	Application No.	Issued (MM/DD/YYYY)
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS	9,132,113	13/794,467	09/15/2015
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS	9,603,825	14/824,688	03/28/2017
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS	9,907,775	15/429,311	03/06/2018
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS IN THE TREATMENT OF NEOPLASMS	9,974,764	14/026,473	05/22/2018
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS FOR THE TREATMENT OF STROKE	9,533,938	14/026,534	01-03-2017
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS FOR THE TREATMENT OF STROKE	10,010,519	15/358,388	07-03-2018
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS FOR THE TREATMENT OF STROKE	9,550,722	14/025,176	01-24-2017
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS	9,636,317	14/027,320	05-02-2017
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS	9,750,713	15/154,100	09-05-2017

Debtor's U.S. Pending Patent Applications

Title	Appl. No.	Publication No.	Filing Date
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS IN THE TREATMENT OF NEOPLASMS	15/938,182	2018-0214409	03-28-2018
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS IN THE TREATMENT OF NEOPLASMS	14/025,206	2016-0332955 and 2015-0072960	09-12-2013
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS IN THE TREATMENT OF NEOPLASMS	16/127,315		09-11-2018
COMPOSITIONS AND METHODS OF	13/745,742	2014-0140979	01-18-2013

USE OF PHORBOL ESTERS FOR THE TREATMENT OF STROKE			
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS FOR THE TREATMENT OF STROKE	15/991,596		05-29-2018
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS	13/745,740	2016-0106698 and 2014-0206762	01-18-2013
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS	15/658,730	2017-0319533	07-25-2017
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS FOR THE TREATMENT OF STROKE	15/371,787	2017-0087112	12-07-2016
COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS	14/025,163	2018-0256529 and 2015-0071874	09-12-2013
PHORBOL ESTER COMPOSITIONS AND METHODS OF USE FOR TREATING OR REDUCING THE DURATION OF CYTOPENIA	14/930,849	2016-0120836	11-03-2015
PHORBOL ESTER COMPOSITIONS AND METHODS OF USE FOR TREATING OR REDUCING THE DURATION OF CYTOPENIA	15/495,163	2017-0224648	04-24-2017

US009132113B2

(12) **United States Patent**
Han et al.

(10) **Patent No.: US 9,132,113 B2**
(45) **Date of Patent: Sep. 15, 2015**

(54) **COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS**

(71) Applicant: **Biosuccess Biotech Co. Ltd.**, Santa Clara, CA (US)

(72) Inventors: **Zheng Tao Han**, Zhengzhou (CN); **Richard L. Chang**, Pinebrook, NJ (US)

(73) Assignee: **BIOSUCCESS BIOTECH COMPANY**, Los Angeles, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/794,467**

(22) Filed: **Mar. 11, 2013**

(65) **Prior Publication Data**

US 2014/0017194 A1 Jan. 16, 2014

Related U.S. Application Data

(63) Continuation of application No. 13/595,072, filed on Aug. 27, 2012, now abandoned, which is a continuation of application No. 12/023,753, filed on Jan. 31, 2008, now abandoned.

(60) Provisional application No. 60/898,810, filed on Jan. 31, 2007.

(51) **Int. Cl.**

A61K 31/215	(2006.01)
A61K 31/573	(2006.01)
A61K 31/60	(2006.01)
A61K 31/225	(2006.01)
A61K 31/55	(2006.01)
A61K 31/606	(2006.01)
A61K 45/06	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 31/225*** (2013.01); ***A61K 31/55*** (2013.01); ***A61K 31/573*** (2013.01); ***A61K 31/606*** (2013.01); ***A61K 45/06*** (2013.01)

(58) **Field of Classification Search**

CPC . A61K 2300/00; A61K 31/216; A61K 31/22; A61K 31/573; A61K 31/55; A61K 45/06; A61K 31/215; A61K 31/225; A61K 31/381; A61K 31/60; A61K 31/23; A61K 31/603; A61K 31/606; C07C 69/21; C07C 69/33

USPC 514/171, 163, 548, 100, 160, 167, 19.2, 514/34, 43, 510, 531, 532, 533, 546, 552; 424/85.2, 94.6, 130.1, 133.1, 142.1, 424/85.7, 94.4, 94.5

See application file for complete search history.

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2011/0245307	A1	10/2011	Alkon	

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Primary Examiner — Savitha Rao

(74) *Attorney, Agent, or Firm* — Sughrue Mion, PLLC

(57) **ABSTRACT**

Methods and compositions containing a phorbol ester or a derivative of a phorbol ester are provided for the treatment of cytopathic diseases. Cytopathic diseases may be caused by a variety means such as viral infections like HIV and AIDS in a mammalian subject. The methods and compositions of the invention are effective for inhibiting de novo HIV infection, upregulating viral expression from latent provirus, inhibiting HIV-induced cytopathic effects, down regulating the HIV receptor, increasing Th1 cytokine expression, and decreasing Th2 cytokine expression. Additional compositions and methods are provided which employ a phorbol ester or derivative compound in combination with at least one additional agent such as those used in HAART protocols or therapeutic agents used to treat opportunistic infections due to HIV in mammalian subjects.

32 Claims, No Drawings

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COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS

RELATED APPLICATIONS

This application claims the benefit as a CONTINUATION of U.S. Continuation patent application Ser. No. 13/595,072, filed Aug. 27, 2012, U.S. patent application Ser. No. 12/023,753, filed Jan. 31, 2008, which is entitled to priority from U.S. Provisional patent application Ser. No. 60/898,810, filed Jan. 31, 2007, each of which priority disclosures is incorporated herein by reference in its entirety.

TECHNICAL FIELD

The present invention relates generally to the field of cytopathic diseases. More specifically, the invention relates to compositions containing and methods of using phorbol esters to treat cytopathic conditions and diseases that cause such cytopathic conditions.

BACKGROUND

Phorbol is a natural, plant-derived organic compound of the tigliane family of diterpenes. It was first isolated in 1934 as a hydrolysis product of *croton* oil derived from the seeds of *Croton tiglium*, a leafy shrub of the Euphorbiaceae family that is native to Southeastern Asia. Various esters of phorbol have important biological properties including the reported ability to mimic diacylglycerols and activate protein kinase C (PKC), modulating downstream cell signaling pathways including the mitogen-activated protein kinase (MAPK) pathways. Phorbol esters are additionally thought to bind to chimaerins, the Ras activator RasGRP, and the vesicle-priming protein Munc-13 (Brose N, Rosenmund C., JCell Sci; 115:4399-411 (2002)). Some phorbol esters also induce nuclear factor-kappa B (NF-κB). The most notable physiological property of phorbol esters is their reported capacity to act as tumor promoters.

12-O-tetradecanoylphorbol-13-acetate (TPA), also called phorbol-12-myristate-13-acetate (PMA), is a phorbol ester used in models of carcinogenesis as an inducer for differentiation and/or apoptosis in multiple cell lines and primary cells. TPA has also been reported to cause an increase in circulating white blood cells and neutrophils in patients whose bone marrow function has been depressed by chemotherapy. (Han Z. T. et al. Proc. Natl. Acad. Sci. 95, 5363-5365 (1998)) and inhibit the HIV-cytopathic effects on MT-4 cells. (Mekkawy S. et al., Phytochemistry 53, 47-464 (2000)). However, due to a variety of factors, including caustic reactions when contacted with the skin and concerns for its potential toxicity, TPA has not been shown to be an effective tool for treating, managing, or preventing HIV or AIDS.

Current therapeutics for cytopathic diseases such as various forms of neoplastic disease and viral diseases such as HIV and AIDS suffer from a number of drawbacks such as insufficient potency and intolerable side effects. For many patients, toxic side effects of diminish their quality of life to such an extent they simply stop taking their medications. For others, therapeutic schedules are so complicated and inconvenient that compliance is limited. Other patients experience excellent results initially, but suffer relapses despite full compliance with therapeutic regimens.

Treatment failure in most HIV cases is attributed to the emergence of resistant strains of HIV. Incomplete viral suppression caused by insufficient drug potency, poor compliance due to complicated drug regimens, and other factors

contribute to this problem. Additionally, during the long period of clinical latency of HIV infection, a subset of quiescent memory CD4 T-cells harbor integrated but transcriptionally silent proviruses. This reservoir protects latent HIV from retroviral therapy and poses a substantial barrier to eradication of HIV in infected patients.

Cancer treatments generally involve a combination of surgery, chemotherapy, hormonal therapy and/or radiation treatment to eradicate neoplastic cells in a patient. However, all of these approaches pose significant drawbacks and added risks such as increased susceptibility to infection. Surgery, for example, may be contraindicated due to the health of a patient. Additionally, it may be difficult to obtain clear margins around a tumor, resulting in some neoplastic tissue being left behind and an increased chance of recurrence of the disease. Almost all current chemotherapeutic agents are toxic, and chemotherapy causes significant side effects including severe nausea, bone marrow depression, and immunosuppression. They also cannot be specifically targeted to cancer cells and therefore may kill healthy cells as well as cancerous ones. Additionally, there are frequently relapsed/refractory neoplasms which are resistant to current therapeutics.

There is clearly a need for new and more effective treatments for individuals suffering from cytopathic disorders, including those caused by neoplastic disease as well as viral infections such as HIV and AIDS.

SUMMARY OF THE EXEMPLARY EMBODIMENTS OF THE INVENTION

The present invention relates to compositions containing and methods of using phorbol esters in the treatment of cytopathic diseases.

In one embodiment, phorbol esters and derivatives of phorbol esters are used to treat cytopathic diseases such as HIV and associated conditions such as AIDS. The compositions and methods of the present invention may accomplish the treatment of HIV and associated conditions such as AIDS by any means possible. In some embodiments, the compositions and methods may modify HIV receptor activity in mammalian subjects. In another embodiment, compositions and methods may decrease the number of latent HIV reservoirs in an HIV-infected subject. In a further embodiment, it may enhance HIV activation in latent pro-viral cells. In additional embodiments, it may inhibit HIV-cytopathic effects.

In another embodiment, compositions containing phorbol esters and phorbol ester derivatives may be used for treating and managing symptoms of HIV and AIDS in mammalian subjects. Targeted symptoms for treatment and management employing the compositions and methods of the invention include, but are not limited to, oral lesions, fatigue, skin thrush, fever, lack of appetite, diarrhea, apthous ulcers, malabsorbtion, thrombocytopenia, weight loss, anemia, lymph node enlargement, susceptibility to and severity of secondary conditions such as *mycobacterium avium* complex, salmonellosis, syphilis, neuroshyphilis, turberculosis (TB), bacillary angiomatosis, aspergillosis, candidiasis, coccidioidomycosis, listeriosis, pelvic inflammatory disease, Burkitt's lymphoma, cryptococcal meningitis, histoplasmosis, Kaposi's sarcoma, lymphoma, systemic non-Hodgkin's lymphoma (NHL), primary CNS lymphoma, cryptosporidiosis, isosporiasis, microsporidiosis, *pneumocystis carinii* pneumonia (PCP), toxoplasmosis, cytomegalovirus (CMV), hepatitis, herpes simplex, herpes zoster, human papiloma virus (HPV,

genital warts, cervical cancer), molluscum contagiosum, oral hairy leukoplakia (OHL), and progressive multifocal leukoencephalopathy (PML).

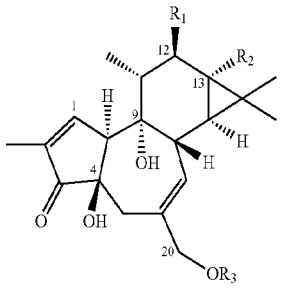
In a further embodiment, compounds containing phorbol esters and derivatives of phorbol esters may be used to treat cytopathic conditions such as neoplastic diseases. Such neoplasms may be malignant or benign. In some embodiments, neoplasms may be solid or non-solid cancers. In other embodiments, the neoplasms may be relapses. In another embodiment, the neoplasms may be refractory. Exemplary neoplasms include, but are not limited to, hematologic malignancies/bone marrow disorders, including, but not limited to, leukemia, including acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic myeloid leukemia blast crisis, myelodysplasia, and myeloproliferative syndrome; lymphoma, including Hodgkins and non-Hodgkins lymphoma; subcutaneous adenocarcinoma; ovarian teratocarcinoma; and prostate cancer. Other neoplastic conditions amenable to treatment using the methods and compositions include other cancer disorders and conditions, including solid tumors of various types, where successful treatment and/or remission will be determined according to conventional methods, such as determining size reduction of solid tumors, and/or histopathological studies to assess growth, stage, metastatic state or potential, presence or expression levels of histological cancer markers, etc.

Compositions and methods herein may additionally be used treat symptoms of neoplastic disease including, but not limited to, anemia; chronic fatigue; excessive or easy bleeding, such as bleeding of the nose, gums, and under the skin; easy bruising, particularly bruising with no apparent cause; shortness of breath; petechiae; recurrent fever; swollen gums; slow healing of cuts; bone and joint discomfort; recurrent infections; weight loss; itching; night sweats; lymph node swelling; fever; abdominal pain and discomfort; disturbances in vision; coughing; loss of appetite; pain in the chest; difficulty swallowing; swelling of the face, neck and upper extremities; a need to urinate frequently, especially at night; difficulty starting urination or holding back urine; weak or interrupted flow of urine; painful or burning urination; difficulty in having an erection; painful ejaculation; blood in urine or semen; frequent pain or stiffness in the lower back, hips, or upper thighs; and weakness.

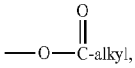
In yet another embodiment, the phorbol esters and derivatives of phorbol esters may be used to modulate cell signaling pathways. Such modulation may have a variety of results, for example, in some embodiments, the use of compositions containing phorbol esters and derivatives of phorbol esters may increase white blood cell counts in mammalian subjects. In another embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the release of Th1 cytokines in mammalian subjects. In a further embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the release of interleukin 2 (IL-2) in mammalian subjects. In an additional embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the release of interferon in mammalian subjects. In yet another embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the rate of ERK phosphorylation.

The invention achieves the foregoing and satisfies additional objects and advantages by providing novel and surprisingly effective methods and compositions for modulating cell signaling pathways and/or treating cytopathic diseases and symptoms of cytopathic diseases or conditions using compositions containing a phorbol ester or derivative composition of the Formula I, below:

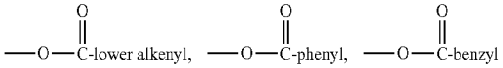
Formula I



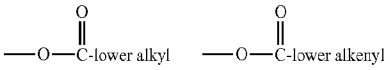
wherein R_1 and R_2 may be hydrogen;



wherein the alkyl group contains 1 to 15 carbon atoms;

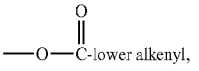


and substituted derivatives thereof and R_3 may be hydrogen or

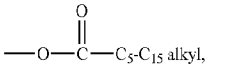


and substituted derivatives thereof.

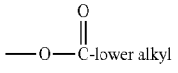
In another embodiment, at least one of R_1 and R_2 are other than hydrogen and R_3 is hydrogen or



and substituted derivatives thereof. In yet another embodiment, either R_1 or R_2 is



the remaining R_1 or R_2 is



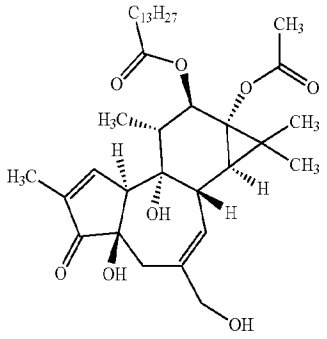
and R_3 is hydrogen.

The alkyl, alkenyl, phenyl and benzyl groups of the formulas herein may be unsubstituted or substituted with halogens, preferably, chlorine, fluorine or bromine; nitro; amino; and/or similar type radicals.

In a further embodiment, the invention achieves these objects and satisfies additional objects and advantages by providing novel and surprisingly effective methods and com-

positions for modulating cell signaling pathways and/or treating cytopathic diseases or conditions associated with cytopathic diseases using an exemplary phorbol ester composition such as 12-O-tetradecanoylphorbol-13-acetate (TPA) of Formula II, below:

Formula II



Useful phorbol esters and related compounds and derivatives within the formulations and methods of the invention include, but are not limited to, other pharmaceutically acceptable active salts of said compounds, as well as active isomers, enantiomers, polymorphs, glycosylated derivatives, solvates, hydrates, and/or prodrugs of said compounds. Exemplary forms of phorbol esters for use within the compositions and methods of the invention include, but are not limited to, phorbol 13-butyrate; phorbol 12-decanoate; phorbol 13-decanoate; phorbol 12,13-diacetate; phorbol 13,20-diacetate; phorbol 12,13-dibenzoate; phorbol 12,13-dibutyrate; phorbol 12,13-didecanoate; phorbol 12,13-dihexanoate; phorbol 12,13-dipropionate; phorbol 12-myristate; phorbol 13-myristate; phorbol 12-myristate-13-acetate (also known as TPA or PMA); phorbol 12,13,20-triacetate; 12-deoxyphorbol 13-angelate; 12-deoxyphorbol 13-angelate 20-acetate; 12-deoxyphorbol 13-isobutyrate; 12-deoxyphorbol 13-isobutyrate-20-acetate; 12-deoxyphorbol 13-phenylacetate; 12-deoxyphorbol 13-phenylacetate 20-acetate; 12-deoxyphorbol 13-tetradecanoate; phorbol 12-tigliate 13-decanoate; 12-deoxyphorbol 13-acetate; phorbol 12-acetate; and phorbol 13-acetate.

In exemplary embodiments, the compositions and methods of the invention employ a phorbol ester compound of Formula I to treat and/or prevent symptoms of cytopathic diseases including, but not limited to, symptoms of HIV and AIDS or other diseases and conditions associated with HIV and AIDS such as opportunistic infections, as well as symptoms of neoplastic diseases or other diseases and conditions associated with neoplastic diseases.

Mammalian subjects amenable to treatment with phorbol esters of Formula I, particularly TPA, according to the methods of the invention include, but are not limited to, subjects with HIV and AIDS, as well as subjects with symptoms, or secondary or opportunistic diseases associated with HIV and AIDS, such as oral lesions, fatigue, skin thrush, fever, lack of appetite, diarrhea, apthous ulcers, malabsorption, thrombocytopenia, weight loss, anemia, lymph node enlargement, *mycobacterium avium* complex, salmonellosis, syphilis, neuroshyphilis, turberculosis (TB), bacillary angiomatosis, aspergillosis, candidiasis, coccidioidomycosis, listeriosis, pelvic inflammatory disease, Burkitt's lymphoma, cryptococcal meningitis, histoplasmosis, Kaposi's sarcoma, lymphoma, systemic non-Hodgkin's lymphoma (NHL), primary CNS lymphoma, cryptosporidiosis, isosporiasis, microsporidiosis, *pneumocystis carinii* pneumonia (PCP), toxoplasmosis, cytomegalovirus (CMV), hepatitis, herpes simplex, herpes zoster, human papiloma virus (HPV, genital warts, cervical cancer), molluscum contagiosum, oral hairy leukoplakia (OHL), and progressive multifocal leukoencephalopathy (PML).

Additional mammalian subjects amenable to treatment with phorbol esters of Formula I, particularly TPA, according to the methods of the present invention include, but are not limited to, subjects suffering from neoplastic diseases including malignant neoplastic diseases such as solid and non-solid cancers. Non-solid cancers may include, hematologic malignancies/bone marrow disorders, including, but not limited to, leukemia, including acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic myeloid leukemia blast crisis, myelodysplasia, myeloproliferative syndrome. Solid cancers may include, but are not limited to, lymphoma, including Hodgkins and non-Hodgkins lymphoma, subcutaneous adenocarcinoma, ovarian teratocarcinoma, and prostate cancer. Subjects amenable to treatment with phorbol esters of Formula I, particularly TPA additionally include those suffering from symptoms of such neoplastic diseases such as, but not limited to, anemia; chronic fatigue; excessive or easy bleeding, such as bleeding of the nose, gums, and under the skin; easy bruising, particularly bruising with no apparent cause; shortness of breath; petechiae; recurrent fever; swollen gums; slow healing of cuts; bone and joint discomfort; recurrent infections; weight loss; itching; night sweats; lymph node swelling; fever; abdominal pain and discomfort; disturbances in vision; coughing; loss of appetite; pain in the chest; difficulty swallowing; swelling of the face, neck and upper extremities; a need to urinate frequently, especially at night; difficulty starting urination or holding back urine; weak or interrupted flow of urine; painful or burning urination; difficulty in having an erection; painful ejaculation; blood in urine or semen; frequent pain or stiffness in the lower back, hips, or upper thighs; and weakness. In some embodiments, such cancers may be relapses or refractory.

These and other subjects are effectively treated, prophylactically and/or therapeutically, by administering to the subject an effective amount of a phorbol ester of Formula I sufficient to prevent or reduce viral load, decrease latent reservoirs of HIV, increase immune responsiveness, increase the release of Th1 cytokines, prevent or reduce symptoms and conditions associated with HIV and AIDS, decrease and/or eliminate neoplastic cells, increase white blood cell counts, induce remission, maintain remission, prevent or reduce symptoms and conditions associated with malignancies and/or increase ERK phosphorylation. Therapeutically useful methods and formulations of the invention will effectively use a phorbol ester of Formula I in a variety of forms, as noted above, including any active, pharmaceutically acceptable salts of said compounds, as well as active isomers, enantiomers, polymorphs, solvates, hydrates, prodrugs, and/or combinations thereof. TPA of formula II is employed as an illustrative embodiment of the invention within the examples herein below.

Within additional aspects of the invention, combinatorial formulations and methods are provided which employ an effective amount of a phorbol ester of Formula I in combination with one or more secondary or adjunctive active agent(s) that is/are combinatorially formulated or coordinately administered with the phorbol ester compound of Formula I to yield an effective response in the subject. Exemplary combinatorial formulations and coordinate treatment methods in the treatment of viral cytopathic diseases such as HIV and AIDS

employ the phorbol ester compound of Formula I in combination with one or more additional, retroviral, HIV or AIDS treating or other indicated secondary or adjunctive therapeutic agents. Such combinatorial formulations and coordinate treatment methods may, for example, follow or be derived from various highly active antiretroviral therapy protocols (HAART protocols) and include regimens such as, but not limited to, two nucleoside analogue reverse transcriptase inhibitors plus one or more protease inhibitor or non-nucleoside analogue reverse transcriptase inhibitor among other combinations. Other combinatorial formulations and coordinate treatment methods may, for example, include treatments for opportunistic infections as well as the compounds for the HAART protocols. The secondary or adjunctive therapeutic agents used in combination with, e.g., TPA, in these embodiments may possess direct or indirect antiviral effects, alone or in combination with, e.g. TPA, may exhibit other useful adjunctive therapeutic activity in combination with, e.g. TPA (such as HIV preventing, HIV treating, HIV reservoir activating, Th1 cytokine increasing activity); or may exhibit adjunctive therapeutic activity useful for treating opportunistic infections associated with HIV alone or in combination with, e.g. TPA.

Useful adjunctive therapeutic agents in these combinatorial formulations and coordinate treatment methods include, for example, protease inhibitors, including, but not limited to, saquinavir, indinavir, ritonavir, nelfinavir, atazanavir, darunavir, fosamprenavir, tipranavir and amprenavir; nucleoside reverse transcriptase inhibitors including but not limited to, zidovudine, didanosine, stavudine, lamivudine, zalcitabine, emtricitabine, tenofovir disoproxil fumarate, AVX754 and abacavir; non-nucleoside reverse transcriptase inhibitors including, but not limited to, nevaripine, delavirdine, calanolide A, TMC125 and efavirenz; combination drugs including, but not limited to, efavirenz/emtricitabine/tenofovir disoproxil fumarate, lamivudine/zidovudine, abacavir/lamivudine, abacavir/lamivudine/zidovudine, emtricitabine/tenofovir disoproxil fumarate, sulfamethoxazole/trimethoprim, and lopinavir/ritonavir; entry and fusion inhibitors, including, but not limited to, enfuvirtide, AMD070, BMS-488043, fozivudine tidoxil, GSK-873,140, PRO140, PRO542, Peptide T, SCH-D, TNX-355, and UK-427,857; treatments for opportunistic infections and other conditions associated with AIDS and HIV including, but not limited to, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, paclitaxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, and valganciclovir; integrase inhibitors including, but not limited to, GS 9137, MK-0518; microbicides, including, but not limited to, BMS-378806, C31G, carbopol 974P, carrageenan, cellulose sulfate, cyanovirin-N, dextran sulfate, hydroxyethyl cellulose, PRO2000, SPL7013, tenofovir, UC-781 and IL-2.

Exemplary combinatorial formulations and coordinate treatment methods in the treatment of neoplastic disease employ the phorbol ester compound of Formula I in combination with one or more additional, neoplastic disease treating or other indicated, secondary or adjunctive therapeutic agents. The secondary or adjunctive therapeutic agents used in combination with, e.g., TPA, in these embodiments may possess direct or indirect chemotherapeutic effects, alone or in combination with, e.g. TPA, may exhibit other useful adjunctive therapeutic activity in combination with, e.g. TPA (such as cytotoxic, anti-inflammatory, NF-κB inhibiting, apoptosis inducing, Th1 cytokine increasing activity); or may exhibit adjunctive therapeutic activity useful for treating neoplasms or associated symptoms alone or in combination with, e.g. TPA.

Useful adjunctive or secondary therapeutic agents in these combinatorial formulations and coordinate treatment methods include doxorubicin, vitamin D3, cytarabine, cytosine arabinoside, daunorubicin, cyclophosphamide, gemtuzumab ozogamicin, idarubicin, mercaptopurine, mitoxantrone, thioguanine, aldesleukin, asparaginase, carboplatin, etoposide phosphate, fludarabine, methotrexate, etoposide, dexamethasone, and choline magnesium trisalicylate. In addition, adjunctive or secondary therapies may be used such as, but not limited to, radiation treatment, hormone therapy and surgery.

The forgoing and additional objects, features, aspects and advantages of the present invention will become apparent from the following detailed description.

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS OF THE INVENTION

Novel methods and compositions have been identified for use in preventing and/or treating cytopathic diseases and conditions in mammalian subjects. In various embodiments, the methods and compositions are effective to prevent or treat HIV and AIDS and related conditions, diseases caused by HIV and AIDS, and/or diseases acquired because of HIV or AIDS infection. In other embodiments, the methods and compositions are effective to prevent or treat neoplastic diseases and symptoms of such diseases. Such neoplastic diseases may or may not be malignant. In some embodiments, the neoplastic diseases may be solid or non-solid cancers. In other embodiments, the cancers may be refractory or relapses.

Formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as novel HIV and AIDS treating compounds.

Formulations and methods provided herein additionally employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof in the treatment of neoplastic diseases.

Viral load decreasing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as novel viral load decreasing agents.

Apoptosis inducing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as chemotherapeutic agents that induce apoptosis in neoplasms.

Remission inducing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically

acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as anti-neoplasm agents.

Immune responsiveness increasing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as immune stimulatory compounds.

Th1 cytokine increasing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as novel Th1 cytokine increasing agents.

A broad range of mammalian subjects, including human subjects, are amenable to treatment using the formulations and methods of the invention. These subjects include, but are not limited to, individuals suffering from cytopathic diseases or conditions including neoplastic diseases and viral cytopathic diseases such as HIV and AIDS.

Subjects amenable to treatment include HIV+ human and other mammalian subjects presenting with oral lesions, fatigue, skin thrush, fever, lack of appetite, diarrhea, apthous ulcers, malabsorption, thrombocytopenia, weight loss, anemia, lymph node enlargement, susceptibility to and severity of secondary conditions such as *mycobacterium avium* complex, salmonellosis, syphilis, neuroshyphilis, turberculosis (TB), bacillary angiomatosis, aspergillosis, candidiasis, coccidioidomycosis, listeriosis, pelvic inflammatory disease, Burkitt's lymphoma, cryptococcal meningitis, histoplasmosis, Kaposi's sarcoma, lymphoma, systemic non-Hodgkin's lymphoma (NHL), primary CNS lymphoma, cryptosporidiosis, isosporiasis, microsporidiosis, *pneumocystis carinii* pneumonia (PCP), toxoplasmosis, cytomegalovirus (CMV), hepatitis, herpes simplex, herpes zoster, human papiloma virus (HPV, genital warts, cervical cancer), molluscum contagiosum, oral hairy leukoplakia (OHL), and progressive multifocal leukoencephalopathy (PML).

Within the methods and compositions of the invention, one or more phorbol ester compound(s) of Formula I as disclosed herein is/are effectively formulated or administered as an agent effective for treating HIV/AIDS and/or related disorders. In exemplary embodiments, TPA is demonstrated for illustrative purposes to be an effective agent in pharmaceutical formulations and therapeutic methods, alone or in combination with one or more adjunctive therapeutic agent(s). The present disclosure further provides additional, pharmaceutically acceptable phorbol ester compounds in the form of a native or synthetic compound, including complexes, derivatives, salts, solvates, isomers, enantiomers, polymorphs, and prodrugs of the compounds disclosed herein, and combinations thereof, which are effective as therapeutic agents within the methods and compositions of the invention in the treatment of HIV/AIDS and related conditions.

Acquired immune deficiency syndrome or acquired immunodeficiency syndrome (AIDS or Aids) is a collection of symptoms and infections resulting from damage to the immune system caused by infection with the human immunodeficiency virus (HIV). The damage to the immune system leaves individuals prone to opportunistic infections and tumors. Although treatments for AIDS and HIV exist to slow the virus's progression and the severity of the symptoms, there is no known cure.

HIV is a retrovirus that primarily infects components of the human immune system such as CD4+ T cells, macrophages and dendritic cells. When CD4+ T cells are destroyed and their total count decreases to below 200 CD4+ T cells/μL of blood or the percentage of CD4+ T-cell as a fraction of the total lymphocytes falls to less than 14%, cellular immunity is lost, leading to AIDS.

It is currently believed that a change in the T_h1 and T_h2 cytokine balance can contribute to immune dysregulation associated with HIV infection. T_h1 cells produce cytokines that stimulate proliferation of cytotoxic T cells. T_h2 cells produce cytokines that are responsible for activation of the humoral immune responses in healthy people. Progression from HIV infection to AIDS is characterized by a decrease in levels of T_h1 cytokines IL-2, IL-12 and IFN-γ with a concomitant increase in levels of T_h2 cytokines IL-4, IL-5 and IL-10. (Clerci, Immunology Today, v. 14, No. 3, p. 107-110, 1993; Becker, Virus Genes 28:1, 5-18 (2004)). Resistance to HIV infection and/or resistance to progression to AIDS may therefore be dependent on a $T_h1 > T_h2$ dominance.

A fraction of CD4+ memory T cells contain integrated transcritpionally inactive proviruses for HIV. These latent reservoirs may be activated to produce active infectious virus following activation by specific antigens or cytokines. The half life of these CD4 memory T cells is at least 44 months making it extremely difficult to eliminate HIV and requiring extended continuation of antiretroviral therapy even when HIV levels in the peripheral blood are undetectable.

Prostratin, 12-deoxyphorbol 13-acetate, a non-tumor promoting phorbol ester, has reportedly shown some effectiveness for inhibiting HIV induced cell killing and viral replication. Prostratin reportedly activated viral expression in latently-infected cell lines, but had little or no effect on chronically-infected cell lines. (Gulakowski, et al., Antiviral Research v. 33, 87-97 (1997); Williams, et al., JBC v. 279, No. 40, P. 42008-42017 (2004)). Prostratin represents a distinct subclass of protein kinase C activators which has unique biological activities that differ from tumor-promoting phorbol esters such as TPA.

Mammalian subjects amenable to treatment with phorbol esters of Formula I, particularly TPA, according to the methods of the present invention additionally include, but are not limited to, mammalian subjects with neoplastic diseases including solid and non-solid cancers, including hematologic malignancies/bone marrow disorders, such as leukemia, including acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic myeloid leukemia blast crisis, myelodysplasia, myeloproliferative syndrome; lymphoma, including Hodgkins and non-Hodgkins lymphoma; subcutaneous adenocarcinoma; ovarian teratocarcinoma; and prostate cancer. In some embodiments, such cancers may be relapses or refractory.

Within the methods and compositions of the invention, one or more phorbol ester compound(s) of Formula I as disclosed herein is/are effectively formulated or administered as an agent effective for treating neoplastic diseases. In exemplary embodiments, TPA is demonstrated for illustrative purposes to be an effective agent in pharmaceutical formulations and therapeutic methods, alone or in combination with one or more adjunctive therapeutic agent(s). The present disclosure further provides additional, pharmaceutically acceptable phorbol ester compounds in the form of a native or synthetic compound, including complexes, derivatives, salts, solvates,

isomers, enantiomers, polymorphs, and prodrugs of the compounds disclosed herein, and combinations thereof, which are effective as therapeutic agents within the methods and compositions of the invention in the treatment of neoplastic diseases and symptoms of such diseases.

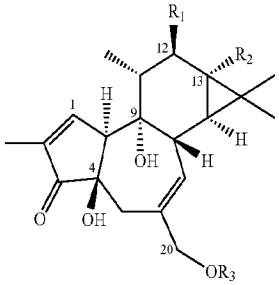
Neoplastic disease is any growth or tumor caused by abnormal and uncontrolled cell division; it may spread to other parts of the body through the lymphatic system or the blood stream. Such growths may be malignant or benign, solid or non-solid.

In some embodiments, the neoplastic diseases may be a hematological neoplasm/bone marrow disorder such as acute myeloid leukemia (AML). AML (also called acute myelogenous leukemia, acute myeloblastic leukemia, acute granulocytic leukemia, and acute nonlymphocytic leukemia) is the most common type of acute leukemia in adults. In AML, stem cells produced by the bone marrow usually develop into a type of immature white blood cell called myeloblasts (or myeloid blasts). In individuals suffering from AML, these myeloblasts do not mature into healthy white blood cells. Additionally, stem cells in individuals with AML may develop into abnormal red blood cells or platelets. The lack of normal blood cells increases incidences of infection, anemia, and easy bleeding. Additionally, the leukemia cells can spread outside the blood to other parts of the body, including the central nervous system (brain and spinal cord), skin, and gums.

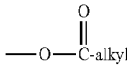
The average age of a patient with AML is over 64 years of age. Patients over the age of 60 treated for AML with standard chemotherapeutics have a remission rate of less than 20%. Additionally, patients who develop AML after an antecedent hematologic disorder or prior leukemogenic chemotherapy/radition therapy have similarly poor outcomes.

Phorbol is a natural, plant-derived polycyclic alcohol of the tigliane family of diterpenes. It was first isolated in 1934 as the hydrolysis product of *croton* oil derived from the seeds of *Croton tiglium*. It is well soluble in most polar organic solvents and in water. Esters of phorbol have the general structure of Formula I, below:

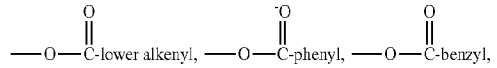
Formula I



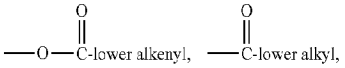
Wherein R_1 and R_2 are selected from the group consisting of hydrogen;



wherein the alkyl group contains 1 to 15 carbon atoms,



and substituted derivatives thereof and R_3 may be hydrogen,



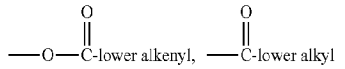
or substituted derivatives thereof.

The term "lower alkyl" or "lower alkenyl" as used herein means moieties containing 1-7 carbon atoms. In the compounds of the Formula I, the alkyl or alkenyl groups may be straight or branched chain. In some embodiments, either or both R_1 or R_2, are a long chain carbon moiety (i.e., Formula I is decanoate or myristate).

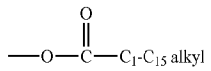
The alkyl, alkenyl, phenyl and benzyl groups of the formulas herein may be unsubstituted or substituted with halogens, preferably, chlorine, fluorine or bromine; nitro; amino and similar type radicals.

Organic and synthetic forms of phorbol esters, including any preparations or extracts from herbal sources such as *croton tiglium*, are contemplated as useful compositions comprising phorbol esters (or phorbol ester analogs, related compounds and/or derivatives) for use within the embodiments herein. Useful phorbol esters and/or related compounds for use within the embodiments herein will typically have a structure as illustrated in Formula I, although functionally equivalent analogs, complexes, conjugates, and derivatives of such compounds will also be appreciated by those skilled in the art as within the scope of the invention.

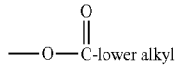
In more detailed embodiments, illustrative structural modifications according to Formula I above will be selected to provide useful candidate compounds for treating and/or preventing HIV and AIDS and/or neoplastic diseases, wherein: at least one of R_1 and R_2 are other than hydrogen and R_3 is selected from the group consisting of hydrogen,



and substituted derivatives thereof. In another embodiment, either R_1 or R_2 is



the remaining R_1 or R_2 is

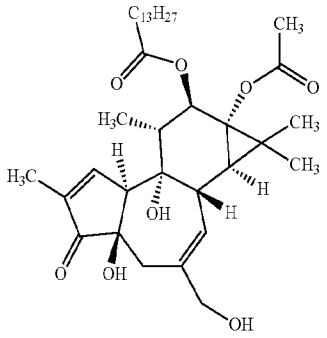


and R_3 is hydrogen.

An exemplary embodiment of a phorbol ester compound of Formula I useful in the treatment of cytopathic diseases such as HIV and AIDS and/or neoplastic diseases, particularly AML, is found in phorbol 12-myristate-13-acetate (also

known as PMA or 12-O-tetradecanoyl-phorbol-13-acetate (TPA)) shown in Formula II, below.

Formula II



Additional useful phorbol esters and related compounds and derivatives within the formulations and methods of the invention include, but are not limited to, other pharmaceutically acceptable active salts of said compounds, as well as active isomers, enantiomers, polymorphs, glycosylated derivatives, solvates, hydrates, and/or prodrugs of said compounds. Further exemplary forms of phorbol esters for use within the compositions and methods of the invention include, but are not limited to, phorbol 13-butyrate; phorbol 12-decanoate; phorbol 13-decanoate; phorbol 12,13-diacetate; phorbol 13,20-diacetate; phorbol 12,13-dibenzoate; phorbol 12,13-dibutyrate; phorbol 12,13-didecanoate; phorbol 12,13-dihexanoate; phorbol 12,13-dipropionate; phorbol 12-myristate; phorbol 13-myristate; phorbol 12,13,20-triacetate; 12-deoxyphorbol 13-angelate; 12-deoxyphorbol 13-angelate 20-acetate; 12-deoxyphorbol 13-isobutyrate; 12-deoxyphorbol 13-isobutyrate-20-acetate; 12-deoxyphorbol 13-phenylacetate; 12-deoxyphorbol 13-phenylacetate 20-acetate; 12-deoxyphorbol 13-tetradecanoate; phorbol 12-tigliate 13-decanoate; 12-deoxyphorbol 13-acetate; phorbol 12-acetate; and phorbol 13-acetate.

Cytopathic disease treating compositions herein comprise HIV- and AIDS-treating compositions comprising an anti-AIDS effective amount of a phorbol ester compound of Formula I, which is effective for prophylaxis and/or treatment of HIV, AIDS, and/or HIV-related symptoms, including opportunistic infections, in a mammalian subject. An "anti-HIV", "anti-AIDS", or "AIDS treating" effective amount of the active compound is therapeutically effective, in a single or multiple unit dosage form, over a specified period of therapeutic intervention, to measurably alleviate one or more symptoms of AIDS in a subject, and/or to alleviate one or more symptom(s) or condition(s) associated with HIV infection in the subject. Within exemplary embodiments, the compositions of the invention are effective in treatment methods to alleviate symptoms of AIDS or other HIV-related conditions in human and other mammalian subjects vulnerable to HIV infection.

Cytopathic disease treating compositions herein additionally may comprise chemotherapeutic compositions comprising an anti-neoplastic effective amount of a phorbol ester or derivative compound of Formula I, which is effective for maintenance and treatment of malignancies or symptoms caused by cancer in a mammalian subject. A "chemotherapeutic", "anti-tumor," "cancer treating", "apoptosis inducing", "remission inducing", "remission maintaining" effective amount of the active compound is therapeutically effective, in a single or multiple unit dosage form, over a specified period of therapeutic intervention, to measurably alleviate one or more symptoms of malignancy in a subject, and/or to alleviate one or more symptom(s) or condition(s) associated with malignancy in the subject. Within exemplary embodiments, the compositions of the invention are effective in treatment methods to alleviate symptoms of neoplastic disease related conditions in human and other mammalian subjects vulnerable to malignancies.

Cytopathic disease treating, including chemotherapeutic and HIV treating, compositions of the invention typically comprise an effective amount or unit dosage of a phorbol ester compound of Formula I, which may be formulated with one or more pharmaceutically acceptable carriers, excipients, vehicles, emulsifiers, stabilizers, preservatives, buffers, and/or other additives that may enhance stability, delivery, absorption, half-life, efficacy, pharmacokinetics, and/or pharmacodynamics, reduce adverse side effects, or provide other advantages for pharmaceutical use. Effective amounts of a phorbol ester compound or related or derivative compound of Formula I (e.g., a unit dose comprising an effective concentration/amount of TPA, or of a selected pharmaceutically acceptable salt, isomer, enantiomer, solvate, polymorph and/or prodrug of TPA) will be readily determined by those of ordinary skill in the art, depending on clinical and patient-specific factors. Suitable effective unit dosage amounts of the active compounds for administration to mammalian subjects, including humans, may range from 10 to 1500 μg, 20 to 1000 μg, 25 to 750 μg, 50 to 500 μg, or 150 to 500 μg. In certain embodiments, the cytopathic disease treating effective dosage of a phorbol ester compound or related or derivative compound of Formula I may be selected within narrower ranges of, for example, 10 to 25 μg, 30-50 μg, 75 to 100 μg, 100 to 250 μg, or 250 to 500 μg. These and other effective unit dosage amounts may be administered in a single dose, or in the form of multiple daily, weekly or monthly doses, for example in a dosing regimen comprising from 1 to 5, or 2 to 3, doses administered per day, per week, or per month. In one exemplary embodiment, dosages of 10 to 30 μg, 30 to 50 μg, 50 to 100 μg, 100 to 250 μg, or 250 to 500 μg, are administered one, two, three, four, or five times per day. In more detailed embodiments, dosages of 50-100 μg, 100-250 μg, 250-400 μg, or 400-600 μg are administered once or twice daily. In a further embodiment, dosages of 50-100 μg, 100-2500 μg, 250-400 μg, or 400-600 μg are administered every other day. In alternate embodiments, dosages are calculated based on body weight, and may be administered, for example, in amounts from about 0.5 μg/sq·m to about 100 μg/sq·m per day, 1 μg/sq·m to about 75 μg/sq·m per day, 1 μg/sq·m to about 50 μg/sq·m per day, 2 μg/sq·m to about 50 μg/sq·m per day, 2 μg/sq·m to about 30 μg/sq·m per day or 3 μg/sq·m to about 30 μg/sq·m per day.

The amount, timing and mode of delivery of compositions of the invention comprising a cytopathic disease treating effective amount of a phorbol ester compound of Formula I (AIDS treating, HIV preventing, HIV treating, HIV reservoir activating, Th1 cytokine increasing, ERK phosphorylation inducing, chemotherapeutic, anti-tumor, cancer treating, remission inducing, remission maintaining, apoptosis inducing effective amount) will be routinely adjusted on an individual basis, depending on such factors as weight, age, gender, and condition of the individual, the acuteness of the cytopathic disease and/or related symptoms, whether the administration is prophylactic or therapeutic, and on the basis of other factors known to effect drug delivery, absorption, pharmacokinetics, including half-life, and efficacy.

An effective dose or multi-dose treatment regimen for the instant cytopathic disease treating (alternatively, "AIDS treat-

ing", "HIV treating", "HIV preventing", "HIV reservoir activating", or "Th1 cytokine increasing", "ERK phosphorylation inducing", "chemotherapeutic", "anti-tumor", "cancer treating", "apoptosis inducing", "remission inducing", "remission maintaining") formulations of the invention will ordinarily be selected to approximate a minimal dosing regimen that is necessary and sufficient to substantially prevent or alleviate the symptoms of the cytopathic disease including AIDS or neoplastic diseases such as cancer and related opportunistic diseases in the subject, and/or to substantially prevent or alleviate one or more symptoms associated with AIDS or neoplastic diseases such as cancer in the subject. A dosage and administration protocol will often include repeated dosing therapy over a course of several days or even one or more weeks or years. An effective treatment regime may also involve prophylactic dosage administered on a day or multi-dose per day basis lasting over the course of days, weeks, months or even years.

Various assays and model systems can be readily employed to determine the therapeutic effectiveness of the treatment of cytopathic diseases. For example in the treatment of HIV or AIDS effectiveness may be demonstrated by a decrease in viral load, an increase in CD4 counts, an increase in CD3 counts, an increase in IL-2 and IFN production, a decrease in IL-4 and IL-10 production, and a decrease or elimination of the symptoms of AIDS among other methods of determining effectiveness known to those of skill in the art.

Effectiveness of the compositions and methods of the invention may be demonstrated, for example, through blood tests for HIV antibodies, viral load, CD4 levels, CD8 counts, and CD3 counts. Normal levels of CD4 are usually between 600 and 1200 per microliter, or 32-68% of lymphocytes. Individuals with a CD4 count of less than 350 have a weakened immune system. Those with a CD4 count of less than 200 are considered to have AIDS. CD8 levels in a healthy individual are generally between 150-1000 per microliter. CD3 levels in a healthy individual are generally between about 885-2270 per microliter. Levels of CD3, CD4 and CD8 cells may be measured, for example, using flow cytometry. Effective amounts of the compositions of the invention will increase levels of CD3, CD4 and CD8 positive cells by at least 10%, 20%, 30%, 50% or greater reduction, up to a 75-90%, or 95% or greater. Effective amounts will also move the CD3, CD4 and CD8 profile of an individual towards the optimal category for each type of glycoprotein.

Individuals may also be evaluated using a $beta_2$-microglobulin ($beta_2$-M) test. $Beta_2$-microglobulin is a protein released into the blood when a cell dies. A rising $beta_2$-M blood level can be used to measure the progression of AIDS. Effective amounts of a composition of the present invention will lead to a decrease or cessation of increase in the amount of $beta_2$-M.

Effectiveness may further be demonstrated using a complete blood count (CBC). The measurements taken in a CBC include a white blood cell count (WBC), a red blood cell count (RBC), the red cell distribution width, the hematocrit, and the amount of hemoglobin. Specific AIDS-related signs in a CBC include a low hematocrit, a sharp decrease in the number of blood platelets, and a low level of neutrophils. An effective amount of a composition of the present invention will increase the levels measured in a complete blood count by 10%, 20%, 30%, 50% or greater increase, up to a 75-90%, or 95% or greater. Effective amounts will also move the blood protein of an individual towards the optimal category for each type of protein.

Effectiveness of the compositions and methods of the invention may also be demonstrated by a decrease in the symptoms of HIV or AIDS including, but not limited to, oral lesions, fatigue, skin thrush, fever, lack of appetite, diarrhea, apthous ulcers, malabsorption, thrombocytopenia, weight loss, anemia, and lymph node enlargement.

Effectiveness of the compositions and methods of the invention may also be demonstrated by a decrease in the susceptibility to and severity of secondary or opportunistic conditions such as *mycobacterium avium* complex, salmonellosis, syphilis, neuroshyphilis, turberculosis (TB), bacillary angiomatosis, aspergillosis, candidiasis, coccidioidomycosis, listeriosis, pelvic inflammatory disease, Burkitt's lymphoma, cryptococcal meningitis, histoplasmosis, Kaposi's sarcoma, lymphoma, systemic non-Hodgkin's lymphoma (NHL), primary CNS lymphoma, cryptosporidiosis, isosporiasis, microsporidiosis, *pneumocystis carinii* pneumonia (PCP), toxoplasmosis, cytomegalovirus (CMV), hepatitis, herpes simplex, herpes zoster, human papiloma virus (HPV, genital warts, cervical cancer), molluscum contagiosum, oral hairy leukoplakia (OHL), and progressive multifocal leukoencephalopathy (PML).

Effectiveness may further be demonstrated by reduction of detectable HIV in the HIV-infected subject; maintaining a normal T cell count; or maintaining normal p24 antigen levels.

Effectiveness in the treatment of neoplastic diseases may also be determined by a number of methods such as, but not limited to, ECOG Performance Scale, the Karnofsky Performance Scale, microscopic examination of blood cells, bone marrow aspiration and biopsy, cytogenetic analysis, biopsy, immunophenotyping, blood chemistry studies, a complete blood count, lymph node biopsy, peripheral blood smear, visual analysis of a tumor or lesion, or any other method of evaluating and/or diagnosing malignancies and tumor progression known to those of skill in the art.

For example, effectiveness of the compositions and methods herein in the treatment of hematologic malignancies/bone marrow disorders may be evaluated using, an absolute neutrophil count (ANC). A normal ANC is between 1,500 to 8,000/mm3. Individuals suffering from hematologic malignancies/bone marrow disorders frequently have an ANC below 1500/mm^3, and may even reach levels below 500/mm^3 Effective amounts of the compositions and methods herein will increase an individual's ANC by 10%, 20%, 30%, 50% or greater increase, up to a 75-90%, or 95% or greater. Effective amounts may increase ANC levels above 1500/mm^3.

Effectiveness of the compositions and methods herein in the treatment of hematologic malignancies/bone marrow disorders may further be evaluated using, for example, a platelet count. A platelet count is normally between 150,000 to 450,000 platelets per microliter (×10−6/Liter). Individuals suffering from hematologic malignancies/bone marrow disorder may have platelet counts below 100,000 per microliter. Effective amounts of the compositions and methods herein will increase an individual's platelet count by 10%, 20%, 30%, 50% or greater increase, up to a 75-90%, or 95% or greater. Effective amounts may increase platelet levels above 100,000 per microliter.

Effectiveness of the compositions and methods herein in the treatment of hematologic malignancies/bone marrow disorders may additionally be evaluated, for example, by measuring the number of myeloblasts. Myeloblasts normally represent less than 5% of the cells in the bone marrow but should not be present in circulating blood. Effective amounts of the compositions and methods herein will decrease the number of myeloblasts by 10%, 20%, 30%, 50% or more, up to a 75-90%, 96% or greater decrease. Effective amounts may decrease myeloblasts to below 5%.

Effectiveness of the compositions and methods herein in the treatment of hematologic malignancies/bone marrow disorders may further be evaluated by examining myeloblasts for the presence of Auer rods. Effective amounts of the compositions of the present invention will decrease the number of Auer rods visible by 10%, 20%, 30%, 50% or more, up to a 75-90%, 96% or greater decrease up to the complete elimination of Auer rods.

Effectiveness of the compositions and methods of the invention may also be demonstrated by a decrease in the symptoms of subjects suffering from neoplastic disease including, but not limited to, anemia; chronic fatigue; excessive or easy bleeding, such as bleeding of the nose, gums, and under the skin; easy bruising, particularly bruising with no apparent cause; shortness of breath; petechiae; recurrent fever; swollen gums; slow healing of cuts; bone and joint discomfort; recurrent infections; weight loss; itching; night sweats; lymph node swelling; fever; abdominal pain and discomfort; disturbances in vision; coughing; loss of appetite; pain in the chest; difficulty swallowing; swelling of the face, neck and upper extremities; a need to urinate frequently, especially at night; difficulty starting urination or holding back urine; weak or interrupted flow of urine; painful or burning urination; difficulty in having an erection; painful ejaculation; blood in urine or semen; frequent pain or stiffness in the lower back, hips, or upper thighs; and weakness.

For each of the indicated conditions described herein, test subjects will exhibit a 10%, 20%, 30%, 50% or greater reduction, up to a 75-90%, or 96% or greater, reduction, in one or more symptom(s) caused by, or associated with, the cytopathic disease, or related diseases or conditions in the subject, compared to placebo-treated or other suitable control subjects.

Within additional aspects of the invention, combinatorial cytopathic disease treating (AIDS treating, HIV preventing, HIV treating, HIV reservoir activating, Th1 cytokine increasing, ERK phosphorylation inducing, apoptosis inducing, chemotherapeutic, anti-tumor, cancer treating, remission inducing, remission maintaining) formulations and coordinate administration methods are provided which employ an effective amount of a phorbol ester compound of Formula I and one or more secondary or adjunctive agent(s) that is/are combinatorially formulated or coordinately administered with the phorbol ester compound of Formula I to yield a combined, multi-active cytopathic disease treating composition or coordinate treatment method.

Exemplary combinatorial formulations and coordinate treatment methods in this context employ the phorbol ester of Formula I in combination with the one or more secondary anti-AIDS agent(s), or with one or more adjunctive therapeutic agent(s) that is/are useful for treatment or prophylaxis of the targeted (or associated) disease, condition and/or symptom(s) in the selected combinatorial formulation or coordinate treatment regimen. For most combinatorial formulations and coordinate treatment methods of the invention, a phorbol ester compound of Formula I or related or derivative compound is formulated, or coordinately administered, in combination with one or more secondary or adjunctive therapeutic agent(s), to yield a combined formulation or coordinate treatment method that is combinatorially effective or coordinately useful to treat HIV/AIDS and/or one or more symptom(s) of a opportunistic or secondary disease or condition in the subject. Exemplary combinatorial formulations and coordinate treatment methods in this context employ a phorbol ester compound of Formula I in combination with one or more secondary or adjunctive therapeutic agents selected from, e.g., protease inhibitors, including, but not limited to, saquinavir, indinavir, ritonavir, nelfinavir, atazanavir, darunavir, fosamprenavir, tipranavir and amprenavir; nucleoside reverse transcriptase inhibitors including but not limited to, zidovudine, didanosine, stavudine, lamivudine, zalcitabine, emtricitabine, tenofovir disoproxil fumarate, AVX754 and abacavir; non-nucleoside reverse transcriptase inhibitors including, but not limited to, nevaripine, delavirdine, calanolide A, TMC125 and efavirenz; combination drugs including, but not limited to, efavirenz/emtricitabine/tenofovir disoproxil fumarate, lamivudine/zidovudine, abacavir/lamivudine, abacavir/lamivudine/zidovudine, emtricitabine/tenofovir disoproxil fumarate, sulfamethoxazole/trimethoprim, and lopinavir/ritonavir; entry and fusion inhibitors, including, but not limited to, enfuvirtide, AMD070, BMS-488043, fozivudine tidoxil, GSK-873,140, PRO140, PRO542, Peptide T, SCH-D, TNX-355, and UK-427,857; treatments for opportunistic infections and other conditions associated with AIDS and HIV including, but not limited to, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, paclitaxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, and valganciclovir; integrase inhibitors including, but not limited to, GS 9137, MK-0518; microbicides, including, but not limited to, BMS-378806, C31G, carbopol 974P, carrageenan, cellulose sulfate, cyanovirin-N, dextran sulfate, hydroxyethyl cellulose, PRO2000, SPL7013, tenofovir, UC-781, and IL-2.

Additional exemplary combinatorial formulations and coordinate treatment methods may additionally employ the phorbol ester of Formula I in combination with one or more secondary anti-tumor agent(s), or with one or more adjunctive therapeutic agent(s) that is/are useful for treatment or prophylaxis of the targeted (or associated) disease, condition and/or symptom(s) in the selected combinatorial formulation or coordinate treatment regimen. For most combinatorial formulations and coordinate treatment methods of the invention, a phorbol ester compound of Formula I or related or derivative compound is formulated, or coordinately administered, in combination with one or more secondary or adjunctive therapeutic agent(s), to yield a combined formulation or coordinate treatment method that is combinatorially effective or coordinately useful to treat neoplastic diseases and one or more symptom(s) of a secondary disease or condition in the subject. Exemplary combinatorial formulations and coordinate treatment methods in this context employ a phorbol ester compound of Formula I in combination with one or more secondary or adjunctive therapeutic agents selected from, e.g., chemotherapeutic agents, anti-inflammatory agents, doxorubicin, vitamin D3, cytarabine, cytosine arabinoside, daunorubicin, cyclophosphamide, gemtuzumab ozogamicin, idarubicin, mercaptopurine, mitoxantrone, thioguanine, aldesleukin, asparaginase, carboplatin, etoposide phosphate, fludarabine, methotrexate, etoposide, dexamethasone, and choline magnesium tri salicylate. In addition, adjunctive or secondary therapies may be used such as, but not limited to, radiation treatment, hormone therapy and surgery.

In certain embodiments the invention provides combinatorial cytopathic disease treating (AIDS treating, HIV preventing, HIV treating, HIV reservoir activating, Th1 cytokine increasing, ERK phosphorylation inducing, apoptosis inducing, chemotherapeutic, anti-tumor, cancer treating, remission inducing, remission maintaining) formulations comprising a phorbol ester and one or more adjunctive agent(s) having

cytopathic disease treating activity. Within such combinatorial formulations, a phorbol ester of Formula I and the adjunctive agent(s) having cytopathic disease treating activity will be present in a combined formulation in cytopathic disease treating (AIDS treating, HIV preventing, HIV treating, HIV reservoir activating, Th1 cytokine increasing, apoptosis inducing, ERK phosphorylation inducing, chemotherapeutic, anti-tumor, cancer treating, remission inducing, remission maintaining) effective amounts, alone or in combination. In exemplary embodiments, a phorbol ester compound of Formula I and a non-phorbol ester agent(s) will each be present in a cytopathic disease treating amount (i.e., in singular dosage which will alone elicit a detectable alleviation of symptoms in the subject). Alternatively, the combinatorial formulation may comprise one or both the phorbol ester compound of Formula I and the non-phorbol ester agents in sub-therapeutic singular dosage amount(s), wherein the combinatorial formulation comprising both agents features a combined dosage of both agents that is collectively effective in eliciting a cytopathic disease or condition symptom alleviating response. Thus, one or both of the phorbol ester of Formula I and non-phorbol ester agents may be present in the formulation, or administered in a coordinate administration protocol, at a sub-therapeutic dose, but collectively in the formulation or method they elicit a detectable decrease in symptoms of cytopathic disease in the subject. For example, in some embodiments, the combinatorial formulation may include one or more compounds from a highly active antiretroviral therapy protocol (HAART protocols) in combination with a phorbol ester, among other combinations. Other combinatorial formulations may, for example, include a phorbol ester and/or compounds effective in treating the opportunistic infections of AIDS as well as compounds from HAART protocols. In other embodiments, the combinatorial formulation may include one or more additional chemotherapeutic agents.

To practice coordinate administration methods of the invention, a phorbol ester compound of Formula I may be administered, simultaneously or sequentially, in a coordinate treatment protocol with one or more of the secondary or adjunctive therapeutic agents contemplated herein. Thus, in certain embodiments a compound is administered coordinately with a non-phorbol ester agent, or any other secondary or adjunctive therapeutic agent contemplated herein, using separate formulations or a combinatorial formulation as described above (i.e., comprising both a phorbol ester compound of Formula I or related or derivative compound, and a non-phorbol ester therapeutic agent). This coordinate administration may be done simultaneously or sequentially in either order, and there may be a time period while only one or both (or all) active therapeutic agents individually and/or collectively exert their biological activities.

In one embodiment, such coordinate treatment methods may, for example, follow or be derived from various highly active antiretroviral therapy protocols (HAART protocols) and include regimens such as, but not limited to, two nucleoside analogue reverse transcriptase inhibitors plus one or more protease inhibitor or non-nucleoside analogue reverse transcriptase inhibitor with a phorbol ester of Formula I, among other combinations. Other coordinate treatment methods may, for example, include a phorbol ester and/or treatments for opportunistic infections as well as compounds from HAART protocols. A distinguishing aspect of all such coordinate treatment methods is that the phorbol ester compound of Formula I exerts at least some activity, which yields a favorable clinical response in conjunction with a complementary AIDS symptom decreasing, or distinct, clinical response provided by the secondary or adjunctive therapeutic agent. Often, the coordinate administration of the phorbol ester compound of Formula I with the secondary or adjunctive therapeutic agent will yield improved therapeutic or prophylactic results in the subject beyond a therapeutic effect elicited by the phorbol ester compound of Formula I, or the secondary or adjunctive therapeutic agent administered alone. This qualification contemplates both direct effects, as well as indirect effects.

Within exemplary embodiments, a phorbol ester compound of Formula I will be coordinately administered (simultaneously or sequentially, in combined or separate formulation(s)), with one or more secondary HIV treating agents, or other indicated or adjunctive therapeutic agents, e.g., selected from, for example, protease inhibitors, including, but not limited to, saquinavir, indinavir, ritonavir, nelfinavir, atazanavir, darunavir, fosamprenavir, tipranavir and amprenavir; nucleoside reverse transcriptase inhibitors including but not limited to, zidovudine, didanosine, stavudine, lamivudine, zalcitabine, emtricitabine, tenofovir disoproxil fumarate, AVX754 and abacavir; non-nucleoside reverse transcriptase inhibitors including, but not limited to, nevaripine, delavirdine, calanolide A, TMC125 and efavirenz; combination drugs including, but not limited to, efavirenz/emtricitabine/tenofovir disoproxil fumarate, lamivudine/zidovudine, abacavir/lamivudine, abacavir/lamivudine/zidovudine, emtricitabine/tenofovir disoproxil fumarate, sulfamethoxazole/trimethoprim, and lopinavir/ritonavir; entry and fusion inhibitors, including, but not limited to, enfuvirtide, AMD070, BMS-488043, fozivudine tidoxil, GSK-873,140, PRO140, PRO542, Peptide T, SCH-D, TNX-355, and UK-427,857; treatments for opportunistic infections and other conditions associated with AIDS and HIV including, but not limited to, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, paclitaxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, and valganciclovir; integrase inhibitors including, but not limited to, GS 9137, MK-0518; microbicides, including, but not limited to, BMS-378806, C31G, carbopol 974P, carrageenan, cellulose sulfate, cyanovirin-N, dextran sulfate, hydroxyethyl cellulose, PRO2000, SPL7013, tenofovir, and UC-781, and IL-2.

In another embodiment, such coordinate treatment methods may, for example, follow or be derived from various chemotherapeutic protocols. Other coordinate treatment methods may, for example, include a phorbol ester and/or treatments for additional symptoms of neoplastic diseases. A distinguishing aspect of all such coordinate treatment methods is that the phorbol ester compound of Formula I exerts at least some activity, which yields a favorable clinical response in conjunction with a complementary neoplastic disease symptom decreasing, or distinct, clinical response provided by the secondary or adjunctive therapeutic agent. Often, the coordinate administration of the phorbol ester compound of Formula I with the secondary or adjunctive therapeutic agent will yield improved therapeutic or prophylactic results in the subject beyond a therapeutic effect elicited by the phorbol ester compound of Formula I, or the secondary or adjunctive therapeutic agent administered alone. This qualification contemplates both direct effects as well as indirect effects.

Within exemplary embodiments, a phorbol ester compound of Formula I will be coordinately administered (simultaneously or sequentially, in combined or separate formulation(s)), with one or more secondary cancer treating agents,

or other indicated or adjunctive therapeutic agents, e.g. doxorubicin, vitamin D3, cytarabine, cytosine arabinoside daunorubicin, cyclophosphamide, gemtuzumab ozogamicin, idarubicin, mercaptopurine, mitoxantrone, thioguanine, aldesleukin, asparaginase, carboplatin, etoposide phosphate, fludarabine, methotrexate, etoposide, dexamethasone, and choline magnesium trisalicylate.

As noted above, in all of the various embodiments of the invention contemplated herein, the cytopathic disease treating methods and formulations may employ a phorbol ester compound of Formula I in any of a variety of forms, including any one or combination of the subject compound's pharmaceutically acceptable salts, solvates, isomers, enantiomers, polymorphs, solvates, hydrates, and/or prodrugs. In exemplary embodiments of the invention, TPA is employed within the therapeutic formulations and methods for illustrative purposes.

The pharmaceutical compositions of the present invention may be administered by any means that achieve their intended therapeutic or prophylactic purpose. Suitable routes of administration for the compositions of the invention include, but are not limited to, conventional delivery routes, devices and methods including injectable methods such as, but not limited to, intravenous, intramuscular, intraperitoneal, intraspinal, intrathecal, intracerebroventricular, intraarterial, subcutaneous and intranasal routes.

The compositions of the present invention may further include a pharmaceutically acceptable carrier appropriate for the particular mode of administration being employed. Dosage forms of the compositions of the present invention include excipients recognized in the art of pharmaceutical compounding as being suitable for the preparation of dosage units as discussed above. Such excipients include, without intended limitation, binders, fillers, lubricants, emulsifiers, suspending agents, sweeteners, flavorings, preservatives, buffers, wetting agents, disintegrants, effervescent agents and other conventional excipients and additives.

If desired, the compositions of the invention can be administered in a controlled release form by use of a slow release carrier, such as a hydrophilic, slow release polymer. Exemplary controlled release agents in this context include, but are not limited to, hydroxypropyl methyl cellulose, having a viscosity in the range of about 100 cps to about 100,000 cps or other biocompatible matrices such as cholesterol.

Some phorbol ester compositions of Formula I of the invention are designed for parenteral administration, e.g. to be administered intravenously, intramuscularly, subcutaneously or intraperitoneally, including aqueous and non-aqueous sterile injectable solutions which, like many other contemplated compositions of the invention, may optionally contain anti-oxidants, buffers, bacteriostats and/or solutes which render the formulation isotonic with the blood of the mammalian subject; and aqueous and non-aqueous sterile suspensions which may include suspending agents and/or thickening agents. The formulations may be presented in unit-dose or multi-dose containers. Additional compositions and formulations of the invention may include polymers for extended release following parenteral administration. The parenteral preparations may be solutions, dispersions or emulsions suitable for such administration. The subject agents may also be formulated into polymers for extended release following parenteral administration. Pharmaceutically acceptable formulations and ingredients will typically be sterile or readily sterilizable, biologically inert, and easily administered. Such polymeric materials are well known to those of ordinary skill in the pharmaceutical compounding arts. Parenteral preparations typically contain buffering agents and preservatives, and injectable fluids that are pharmaceutically and physiologically acceptable such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like. Extemporaneous injection solutions, emulsions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as described herein above, or an appropriate fraction thereof, of the active ingredient(s).

In more detailed embodiments, compositions of the invention may comprise a phorbol ester compound of Formula I encapsulated for delivery in microcapsules, microparticles, or microspheres, prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly (methylmethacylate) microcapsules, respectively; in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules); or within macroemulsions.

As noted above, in certain embodiments the methods and compositions of the invention may employ pharmaceutically acceptable salts, e.g., acid addition or base salts of the above-described phorbol ester compounds of Formula I and/or related or derivative compounds. Examples of pharmaceutically acceptable addition salts include inorganic and organic acid addition salts. Suitable acid addition salts are formed from acids which form non-toxic salts, for example, hydrochloride, hydrobromide, hydroiodide, sulphate, hydrogen sulphate, nitrate, phosphate, and hydrogen phosphate salts. Additional pharmaceutically acceptable salts include, but are not limited to, metal salts such as sodium salts, potassium salts, cesium salts and the like; alkaline earth metals such as calcium salts, magnesium salts and the like; organic amine salts such as triethylamine salts, pyridine salts, picoline salts, ethanolamine salts, triethanolamine salts, dicyclohexylamine salts, N,N'-dibenzylethylenediamine salts and the like; organic acid salts such as acetate, citrate, lactate, succinate, tartrate, maleate, fumarate, mandelate, acetate, dichloroacetate, trifluoroacetate, oxalate, and formate salts; sulfonates such as methanesulfonate, benzenesulfonate, and p-toluenesulfonate salts; and amino acid salts such as arginate, asparginate, glutamate, tartrate, and gluconate salts. Suitable base salts are formed from bases that form non-toxic salts, for example aluminum, calcium, lithium, magnesium, potassium, sodium, zinc and diethanolamine salts.

Other detailed embodiments, the methods and compositions of the invention for employ prodrugs of phorbol esters of Formula I. Prodrugs are considered to be any covalently bonded carriers which release the active parent drug in vivo. Examples of prodrugs useful within the invention include esters or amides with hydroxyalkyl or aminoalkyl as a substituent, and these may be prepared by reacting such compounds as described above with anhydrides such as succinic anhydride.

The invention disclosed herein will also be understood to encompass methods and compositions comprising phorbol esters of Formula I using in vivo metabolic products of the said compounds (either generated in vivo after administration of the subject precursor compound, or directly administered in the form of the metabolic product itself). Such products may result for example from the oxidation, reduction, hydrolysis, amidation, esterification and the like of the administered compound, primarily due to enzymatic processes. Accordingly, the invention includes methods and compositions of the invention employing compounds produced by a process comprising contacting a phorbol ester

compound of Formula I with a mammalian subject for a period of time sufficient to yield a metabolic product thereof. Such products typically are identified by preparing a radiolabelled compound of the invention, administering it parenterally in a detectable dose to an animal such as rat, mouse, guinea pig, monkey, or to man, allowing sufficient time for metabolism to occur and isolating its conversion products from the urine, blood or other biological samples.

The invention disclosed herein will also be understood to encompass diagnostic compositions for diagnosing the risk level, presence, severity, or treatment indicia of, or otherwise managing cytopathic diseases including, but not limited to, neoplastic disesases including malignant neoplastic diseases such as leukemia, and an AIDS or a related disease or condition in a mammalian subject, comprising contacting a labeled (e.g., isotopically labeled, fluorescent labeled or otherwise labeled to permit detection of the labeled compound using conventional methods) phorbol ester compound of Formula I to a mammalian subject (e.g., to a cell, tissue, organ, or individual) at risk or presenting with one or more symptom(s) of cancer and/or AIDS, and thereafter detecting the presence, location, metabolism, and/or binding state (e.g., detecting binding to an unlabeled binding partner involved in HIV receptor physiology/metabolism or malignant cell receptor physiology/metabolism) of the labeled compound using any of a broad array of known assays and labeling/detection methods. In exemplary embodiments, a phorbol ester compound of Formula I is isotopically-labelled by having one or more atoms replaced by an atom having a different atomic mass or mass number. Examples of isotopes that can be incorporated into the disclosed compounds include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, and ^{36}Cl, respectively. The isotopically-labeled compound is then administered to an individual or other subject and subsequently detected as described above, yielding useful diagnostic and/or therapeutic management data, according to conventional techniques.

EXAMPLES

The experiments described below demonstrate novel and powerful uses for phorbol esters and derivative compounds as HIV treating drugs that can effectively decrease the symptoms of AIDS. In exemplary clinical trials, individuals who were unresponsive to traditional treatments for HIV and AIDS were responsive to treatments with TPA. The treatment with TPA was allowed as "compassionate" and recovery of some patients was considered life-saving according to the attending physicians. The experiments described below additionally demonstrate the usefulness of phorbol esters and derivative compounds in the treatment of neoplastic diseases. These and additional findings are further expanded and elucidated within the following examples.

Example I

Effect of TPA on the Peripheral White Blood Cells (WBC) and Hemoglobin (Hb) Counts in 5180 Cell-Injected Mice

Sarcoma 180 (S180) cells were injected into Kwen-Ming mice. On the third day, the mice were given TPA interperitoneally (i.p.). at 50, 100 or 200 μg/kg/day for 7 days. On the second day after the treatment was completed, blood samples were taken from the tails of the treated mice for WBC and Hb analyses. The WBC counts for the treated groups (50, 100, or 200 ug/kg/day for 7 days) were 16.1±7.4, 18.7±.3.0 and $20.7\pm.3.4\times10^{9}$/L, respectively; the WBC count for the control group was $13.6\pm1.8\times10^{9}$/L. The Hb of the treated groups were 136±11, 149±12 and 149±10 g/L, and the Hb of the control group was 134+-15 g/L. The results indicate that i.p. injection of TPA could increase the peripheral WBC counts in mice in a dose-dependent manner, whereas the Hb levels were not greatly affected in TPA treated mice when compared to the control mice.

Example II

Dose Ranging Study

Due to the strong local irritation caused by TPA application, TPA was given to patients by intravenous (i.v.) infusion. TPA solution in a sterile syringe was injected into 200 ml of sterile saline and mixed well for i.v. infusion.

The Toxicity and Side Effects of Different TPA Doses Administered Clinically:

(1) TPA Given at 1 mg/Patient/Week:

One mg TPA in solution was mixed well with 200 ml of sterile saline for intravenous infusion which was completed in 1 h at the rate of 16 μg/min. One hour after TPA administration, patients started to have chills which lasted for about 30 min, followed by fever, (the patients' temperature reached 37.5-39.5° C. which lasted for 3-5 h, then returned to normal) with light to heavy perspiration. The above symptoms could be alleviated by giving the patients glucocorticoids. TPA at this dose caused a minority of patients to bleed, several patients suffered for a short period of time difficulty in breathing, and Hb was detected in the urine. However, these side effects were short lived and reversible. The cardiac, hepatic, renal and pulmonary functions were all found to be normal.

(2) TPA Given at 0.5 mg/Patient×2/Week: (Two Doses a Week)

0.5 mg of TPA in solution was mixed well with 200 ml of saline for intravenous infusion which was completed in 1 h at the rate of 8 mg/min. The reactions after administration were similar to that of the 1 mg TPA dosage, but to a lesser extent than the 1 mg dose. The patients tolerated the lower dose more easily. Occasionally, Hb was detected in patients' urine. Difficulty in breathing was not observed. The cardiac, hepatic, renal and pulmonary functions were all normal.

(3) TPA Given at 0.25 mg/Patient×4/Week:

0.25 mg of TPA in solution was mixed well with 200 ml of saline for intravenous infusion which was completed in 1 h at the rate of 4 μg/min. After administration, symptoms such as chills and fever were also observed, but to a much lesser extent than with the higher dosages. No Hb was detected in the urine, and no patient suffered difficulty in breathing. The cardiac, hepatic, renal and pulmonary functions were all normal.

Example III

First Clinical Study of HIV+ Patients Treated with TPA

Twelve symptomatic patients (five males and seven females) between the ages of 35 to 52 all of whom were infected with HIV in 1995 through blood transfusion and were refractory to standard treatments for HIV were treated with TPA. Each patient was administered a weight adjusted dosage of TPA (75 μg/sq m) in 200 ml of sterile saline by i.v. over one hour. This dose was administered once daily for the first three days of treatment. Each patient was then given this

dose every other day for days 4 to 18 followed by a six month rest period prior to a second course of treatment according to the same protocol.

Blood samples were gathered prior to administration of the first dose of TPA and on days 4 and 40 of the treatment cycle. Levels of CD3, CD4 and CD8 in peripheral blood were measured using monoclonal antibodies (Becton Dickson Scientific Co., Franklin Lakes, N.J.) and a flow cytometer (B.D. Bioscience, San Diego, Calif.).

As can be seen in Table 1, no consistent change or correlation was observed in CD3, CD4, or CD8 levels.

TABLE ONE

CD_4 CD_8 CD_3 TEST RESULTS OF TWELVE HIV PATIENTS

PATIENT NO	TEST TIME	CD_4	CD_8	CD_3
01-1	Before TPA	3	196	341
01-2	Four days after TPA	3	180	299
01-3	Forty two days after TPA	2	111	203
02-1	Before TPA	26	614	687
02-2	Four days after TPA	105	<2000	2616
02-3	Forty two days after TPA	54	700	799
03-1	Before TPA	32	524	543
03-2	Four days after TPA	36	366	427
03-3	Forty two days after TPA	33	374	424
04-1	Before TPA	173	735	975
04-2	Four days after TPA	123	770	941

TABLE ONE-continued

CD_4 CD_8 CD_3 TEST RESULTS OF TWELVE HIV PATIENTS

PATIENT NO	TEST TIME	CD_4	CD_8	CD_3
04-3	Forty two days after TPA	44	493	581
05-1	Before TPA	106	1556	1646
05-2	Four days after TPA	119	1330	1282
05-3	Forty two days after TPA	191	1429	1643
06-1	Before TPA	232	865	1221
06-2	Four days after TPA	179	570	808
06-3	Forty two days after TPA	49	429	537
07-1	Before TPA	10	988	1022
07-2	Four days after TPA	7	570	598
07-3	Forty two days after TPA	1	139	146
08-1	Before TPA	524	725	1332
08-2	Four days after TPA	318	355	739
08-3	Forty two days after TPA	241	527	858
09-1	Before TPA	442	1021	1479
09-2	After TPA	663	<2000	2920
10-1	Before TPA	407	328	778
10-2	After TPA	445	591	1077
11-1	Before TPA	40	322	373
11-2	After TPA	131	724	874
12-1	Before TPA	84	256	375
12-2	After TPA	78	268	362

As can be seen in Table 2, below, there were similarly inconsistent results in the change of viral load with five patients having an increase in HIV and no change or a reduction in seven others.

TABLE TWO

BLOOD HIV COUNT OF THE TWELVE PATIENTS BEFORE DURING AND AFTER THE TPA TREATMENT

PATIENT NO	TEST TIME	RESULTS (copies/ml)	LOG VALUE	FOOT NOTE
01-1	3 days before TPA	3.36×10^5	5.526	
01-2	4 days after initial TPA	1.41×10^4	6.151	
01-3	15 days after initial TPA	2.02×10^4	4.306	
01-4	25 days after initial TPA	2.60×10^4	4.416	
02-1	3 days before TPA	9.97×10^4	4.999	
02-2	4 days after initial TPA	7.92×10^6	6.899	
02-3	15 days after initial TPA	6.33×10^6	6.801	
02-4	25 days after initial TPA	8.72×10^6	6.941	
03-1	3 days before TPA	3.77×10^5	5.577	
03-2	4 days after initial TPA	8.13×10^4	4.910	
03-3	15 days after initial TPA	6.11×10^3	3.786	
03-4	25 days after initial TPA	8.59×10^5	5.934	
04-1	3 days before TPA	1.11×10^6	6.045	
04-2	4 days after initial TPA	1.75×10^7	7.243	
04-3	15 days after initial TPA	1.11×10^6	6.614	
04-4	25 days after initial TPA	1.21×10^4	4.084	
05-1	3 days before TPA	2.49×10^4	6.637	
05-2	4 days after initial TPA	9.42×10^5	5.974	
05-3	15 days after initial TPA	2.34×10^7	7.369	
05-4	25 days after initial TPA	5.56×10^6	6.745	
06-1	3 days before TPA	4.57×10^5	5.660	
06-2	4 days after initial TPA	1.44×10^4	4.160	
06-3	15 days after initial TPA	1.88×10^5	5.274	
06-4	7 days after TPA	2.28×10^6	6.357	
07-1	3 days before TPA	2.40×10^5	5.623	
07-2	4 days after initial TPA	1.51×10^5	5.179	
07-3	15th day during TPA	9.74×10^4	4.988	
07-4	25 days after initial TPA	5.30×10^3	3.724	
08-1	3 days before TPA	8.02×10^5	5.904	
08-2	4 days after initial TPA	9.09×10^5	5.959	
08-3	15 days after initial TPA	5.46×10^6	6.737	
08-4	25 days after initial TPA	7.77×10^6	6.890	
09-1	3 days before TPA	undetectable		
09-2	25 days after TPA	undetectable		
10-1	3 days before TPA	1.51×10^4	4.180	Sample taken from the
10-2	25 days after initial TPA	2.79×10^4	4.446	second cycle treatment
11-1	3 days before TPA	1.59×10^5	5.201	Sample taken from the

TABLE TWO-continued

BLOOD HIV COUNT OF THE TWELVE PATIENTS BEFORE DURING AND AFTER THE TPA TREATMENT

PATIENT NO	TEST TIME	RESULTS (copies/ml)	LOG VALUE	FOOT NOTE
11-2	25 days after initial TPA	1.25×10^5	5.096	second cycle treatment
12-1	3 days before TPA	1.32×10^4	4.122	Sample taken from the
12-2	25 days after initial TPA	6.27×10^3	3.798	second cycle treatment

Despite the lack of correlation with viral and CD3, CD4 and CD9 levels, eleven of the patients showed significant improvement following treatment. Eight patients became symptom free and five of them have been in remission for 6 to 12 months. Three additional patients had a decrease in symptoms.

Example IV

Second Clinical Study of HIV+ Patients Treated with TPA

Nine of the patients in Example III were given a second treatment of TPA. Of these nine, seven were asymptomatic at the beginning of the second trial. A tenth patient (patient #2a) who was symptomatic and had not previously been treated with TPA was added to the study. Each patient was administered a weight adjusted dosage of TPA (75 μg/sq m) in 200 ml of sterile saline intravenously over one hour. This dosage was given to each patient once a day for ten consecutive days followed by a rest period of ten days for three cycles and a total of 30 doses of TPA. Patients 5a, 6a, and 8a stopped taking anti-AIDS drugs one month prior to beginning the TPA treatment and beginning again one month after the third cycle. Patients 1-4-a, 7a, and 9a-10a continued taking anti-AIDS drugs throughout the treatment.

Blood samples were taken three days prior to starting treatment, after completing the first 10 day cycle of TPA infusion and again after the last TPA infusion and CD3, CD4, CD8, WBC, RBC, HGB and platelets were measured.

As shown in Table 3, there was an increase in CD3 in all patients after the first and third infusion with TPA with the highest value occurring after the third cycle, with the exception of two patients (5a & 10a). There was a trend for increases in the CD8 and in CD4. These results suggest a strengthening of the immune systems with TPA treatment. Varied results were obtained in the HIV count (Table 4). The HIV measurements in some of the patients were below the limits of detection of the method (less than 200) while it increased somewhat in others. There was normal variation in the measurement of WBC, RBC, HGB and platelets (Table 5).

TABLE THREE

CD_4 CD_8 CD_3 TEST RESULTS OF 10 HIV PATIENTS

PATIENT NO	TEST TIME	CD_4	CD_8	CD_3
01-1	Before TPA	5	576	1071
01-2	After first 10-day TPA infusion cycle	7	907	1323
01-3	After third 10-day TPA infusion cycle	19	1129	2037
02a-1	Before TPA	26	307	339
02a-2	After first 10-day TPA infusion cycle	76	335	476
02a-3	After third 10-day TPA infusion cycle	137	543	625
03a-1	Before TPA	295	571	870

TABLE THREE-continued

CD_4 CD_8 CD_3 TEST RESULTS OF 10 HIV PATIENTS

PATIENT NO	TEST TIME	CD_4	CD_8	CD_3
03a-2	After first 10-day TPA infusion cycle	460	729	1200
03a-3	After third 10-day TPA infusion cycle	1002	980	2033
04a-1	Before TPA	152	672	896
04a-2	After first 10-day TPA infusion cycle	189	584	823
04a-3	After third 10-day TPA infusion cycle	205	916	1193
05a-1	Before TPA	92	1097	1175
05a-2	After first 10-day TPA infusion cycle	91	1507	1598
05a-3	After third 10-day TPA infusion cycle	94	1127	1257
06a-1	Before TPA	230	378	669
06a-2	After first 10-day TPA infusion cycle	285	429	758
06a-3	After third 10-day TPA infusion cycle	276	466	938
07a-1	Before TPA	567	1736	2258
07a-2	After first 10-day TPA infusion cycle	729	>2000	3148
07a-3	After third 10-day TPA infusion cycle	786	>2000	3347
08a-1	Before TPA	361	569	1023
08a-2	After first 10-day TPA infusion cycle	519	547	1143
08a-3	After third 10-day TPA infusion cycle	495	733	1295
09a-1	Before TPA	101	533	672
09a-2	After first 10-day TPA infusion cycle	136	574	712
09a-3	After third 10-day TPA infusion cycle	100	1221	1317
10a-1	Before TPA	49	178	240
10a-2	After first 10-day TPA infusion cycle	74	261	333
10a-3	After third 10-day TPA infusion cycle	63	208	308

TABLE FOUR

BLOOD HIV COUNT OF THE TEN PATIENTS BEFORE DURING AND AFTER THE THREE TEN-DAY TPA INFUSION

PATIENT NO	TEST TIME	RESULTS (copies/ml)	LOG VALUE
01-1	3 days before TPA	4.57×10^6	6.660
01-2	after first cycle TPA infusion	2.99×10^5	5.475
01-3	after third cycle TPA infusion	9.41×10^5	5.973
02a-1	3 days before TPA	2.71×10^5	5.433
02a-2	after first cycle TPA infusion	3.09×10^5	5.490
02a-3	after third cycle TPA infusion	9.24×10^5	5.966
03a-1	3 days before TPA	undetectable	—
03a-2	after first cycle TPA infusion	lower the 500	2.371
03a-3	after third cycle TPA infusion	9.55×10^3	3.980
04a-1	3 days before TPA	lower than 500	2.312
04a-2	after first cycle TPA infusion	undetectable	—
04a-3	after third cycle TPA infusion	2.38×10^3	3.376
05a-1	3 days before TPA	undetectable	—
05a-2	after first cycle TPA infusion	undetectable	—
05a-3	after third cycle TPA infusion	undetectable	—
06a-1	3 days before TPA	undetectable	—
06a-2	after first cycle TPA infusion	undetectable	—
06a-3	after third cycle TPA infusion	undetectable	—
07a-1	3 days before TPA	undetectable	—
07a-2	after first cycle TPA infusion	undetectable	—
07a-3	after third cycle TPA infusion	undetectable	—
08a-1	3 days before TPA	1.13×10^4	4.054
08a-2	after first cycle TPA infusion	6.68×10^4	4.825
08a-3	after third cycle TPA infusion	6.20×10^4	4.792
09a-1	3 days before TPA	1.38×10^5	5.139

TABLE FOUR-continued

BLOOD HIV COUNT OF THE TEN PATIENTS BEFORE DURING AND AFTER THE THREE TEN-DAY TPA INFUSION

PATIENT NO	TEST TIME	RESULTS (copies/ml)	LOG VALUE
09a-2	after first cycle TPA infusion	1.65×10^5	5.217
09a-3	after third cycle TPA infusion	2.35×10^5	5.371
10a-1	3 days before TPA	7.20×10^5	5.857
10a-2	after first cycle TPA infusion	2.82×10^5	5.450
10a-3	after third cycle TPA infusion	1.86×10^5	5.270

TABLE FIVE

PERIPHERY BLOOD COUNT OF THE TEN PATIENTS BEFORE AND AFTER THE TPA THREE 10-DAY TREATMENT

PATIENT NO	TEST TIME	WBC ($\times 10^9$/L)	RBC ($\times 10^{12}$/L)	HGB (g/L)	PLt ($\times 10^9$/L)
01-1	Before TPA	2.3	2.55	92	199
01-2	After first 10-day TPA infusing	4.4	2.61	99	325
01-3	After third 10-day TPA infusing	6.1	2.91	102	182
02a-1	Before TPA	5.7	2.44	114	227
02a-2	After first 10-day TPA infusing	3.7	2.14	88	238
02a-3	After third 10-day TPA infusing	11.1	2.52	100	124
03a-1	Before TPA	7.8	4.04	147	309
03a-2	After first 10-day TPA infusing	9.8	3.83	1.38	338
03a-3	After third 10-day TPA infusing	13.6	4.54	140	549
04a-1	Before TPA	3.9	3.34	127	232
04a-2	After first 10-day TPA infusing	3.6	2.92	107	306
04a-3	After third 10-day TPA infusing	9.2	2.85	105	105
05a-1	Before TPA	5.1	3.54	146	243
05a-2	After first 10-day TPA infusing	5.7	3.46	1.35	315
05a-3	After third 10-day TPA infusing	10.1	3.61	144	130
06a-1	Before TPA	5.0	4.21	171	198
06a-2	After first 10-day TPA infusing	4.2	3.48	142	256
06a-3	After third 10-day TPA infusing	6.5	3.66	154	169
07a-1	Before TPA	6.6	3.62	102	306
07a-2	After first 10-day TPA infusing	6.0	3.76	143	258
07a-3	After third 10-day TPA infusing	6.0	3.92	123	293
08a-1	Before TPA	3.1	4.03	125	116
08a-2	After first 10-day TPA infusing	4.3	3.86	128	221
08a-3	After third 10-day TPA infusing	6.8	4.19	128	138
09a-1	Before TPA	3.5	1.43	41	114
09a-2	After first 10-day TPA infusing	2.6	1.99	57	214
09a-3	After third 10-day TPA infusing	4.0	2.33	67	170
10a-1	Before TPA	2.6	2.65	78	297
10a-2	After first 10-day TPA infusing	2.9	2.58	92	187
10a-3	After third 10-day TPA infusing	7.0	4.31	130	138

Of nine patients previously treated with TPA in the first clinical study, only one (#9a) presented with some AIDS symptoms prior to the start of the second clinical study. Following treatment with three cycles of TPA in the second study, this patient and another (#2a), who had never been treated with TPA, experienced a disappearance of AIDS symptoms and both became sufficiently well to resume their normal activities. The other eight patients began the study without AIDS symptoms and were symptom free at the end of the study. All patients remain under observation. Treatment with anti-AIDS drugs continues uninterrupted.

As can be seen in Table 4, there was an increase in all patients in the CD 3, 4 and 8 levels with the most striking and consistent increases in CD3 levels. The viral load of HIV varied. It was undetectable in three patients (<200); it increased somewhat in six others and was reduced in one.

Example V

Third Clinical Study of HIV+ Patients Treated with TPA

Six patients, two males and four females between the ages of 37 and 52 years of age (Patients #13-18), were treated with TPA. Four of these patients previously received TPA treatment in combination with anti-HIV drugs in the two previous clinical studies. The two remaining patients had never been treated with TPA, but had previously received anti-HIV drug regimens. All treatments were stopped three days prior to the initiation of the third clinical study and were not resumed until 60 days after completion of the TPA treatment. The resumption of the standard HIV treatments was required by local health authorities.

Each patient in the study received 150 μg of TPA in 200 ml of sterile saline by intravenous infusion over a 1.5 to 2 hour period daily for 60 days for a total administered dose of 9.0 mg. Following completion of the 60 days of TPA therapy, these patients remained under observation for an additional 60 days though the received no further treatment.

CD3, CD4 and CD8 levels in peripheral blood were quantitated prior to starting treatment, and again at 30 and 60 days using flow cytometry and the appropriate antibodies obtained from B.D. Bioscience, San Diego, Calif. Viral load was determined using conventional methods at Kuang Ann men Hospital, Beijing, China. Patients RBC, WBC, platelets and hemoglobin levels were also measured.

As can be seen in Table 6, the viral load in the six patients was either low or undetectable at the beginning of the trial and remained low throughout the clinical trial period despite the discontinuation of traditional antiretroviral therapy. Addi-

tionally, there was no rebound in viral levels 6 to 15 days after stopping antiretroviral treatment as previously reported as occurring in patients with a plasma viral load below 50 HIV copies per ml. (Harrigan et al., AIDS 13, F59-F62 (1999). The CD3, CD4 and CD8 levels were variable and inconclusive.

TABLE SIX

STUDY 3
$CD_4CD_8CD_3$ AND HIV LOAD RESULTS OF 6 PATIENTS

PATIENT#	*TEST TIME	CD3	CD4	CD8	**HIV (copies/ml)
13	1	3500	1135	>2000	undetectable
	2	2771	735	1938	0.533
	3	2689	721	1897	0.133
14	1	1415	677	664	0.374
	2	1522	613	796	0.353
	3	902	369	485	0.038
15	1	759	9	542	0.533
	2	1865	8	1408	1.99
	3	2099	11	1507	undetectable
16	1	1368	128	1166	undetectable
	2	1477	105	1318	1.28
	3	1305	46	1220	0.012
17	1	428	95	297	0.002
	2	594	112	424	0.152
	3	317	31	246	0.056
18	1	1041	392	457	undetectable
	2	703	229	343	0.174
	3	579	165	290	undetectable

*Test time:
1. Before TPA
2. Thirty days after TPA
3. Sixty days after TPA
**All figures are in the million

White blood cells (WBC), red blood cells (RBC), hemoglobin (Rb) and platelets (PLt) were measured prior to starting TPA treatment, 15, 30, 45 and 60 days after starting TPA treatment and 30 days after stopping TPA treatment. As can be seen in Table 7, most values were within the normal range.

The patients involved in the third clinical study experienced no viral load rebound as typically seen when antiretroviral therapies are discontinued. They additionally had no recurrence of AIDS symptoms during the 120 day observation and treatment period, felt normal and were able to conduct their usual life activities.

TABLE SEVEN

STUDY 3
PERIPHERY BLOOD PROFILE OF 6 PATIENTS

PATIENT#	*TEST TIME	WBC ($\times 10^9$/L) ×	RBC ($\times 10^{12}$/L)	Rb (g/L)	PLt ($\times 10^9$/L)
13	1	9	3.75	139	246
	2	9	3.88	140	240
	3	8.9	4.35	148	275
	4	4.6	3.9	125	304
	5	8.8	4.55	126	221
	6	7.5	4.55	130	272
14	1	4.2	4.16	111	188
	2	4.1	4.03	114	169
	3	5.9	4.48	116	232
	4	3.9	4.44	109	152
	5	4.4	4.31	96	227
	6	6.5	4.4	104	193
15	1	5.9	3.67	110	397
	2	5	3.41	101	219
	3	5.2	3.83	113	247
	4	6.2	4.13	110	262
	5	6.2	4.04	99	239
	6	8.4	3.9	110	278

TABLE SEVEN-continued

STUDY 3
PERIPHERY BLOOD PROFILE OF 6 PATIENTS

PATIENT#	*TEST TIME	WBC ($\times 10^9$/L) ×	RBC ($\times 10^{12}$/L)	Rb (g/L)	PLt ($\times 10^9$/L)
16	1	6	3.62	144	297
	2	8.1	3.65	142	415
	3	4.3	4.03	145	345
	4	4.6	3.86	124	291
	5	5.1	4.1	123	276
	6	3.8	4.71	144	224
17	1	5.5	3.06	124	242
	2	6.4	2.98	118	151
	3	4	3.2	121	177
	4	3.9	3.49	116	131
	5	7.7	3.34	99	121
	6	4.8	3.42	100	178
18	1	7.4	3.91	156	240
	2	8.1	3.69	141	208
	3	4.5	4.32	154	228
	4	4.9	4.14	131	149
	5	3.5	4.56	136	222
	6	NA	NA	NA	NA

*Test time:
1. Before TPA
2. Fifteen days after TPA
3. Thirty days after TPA
4. Forty five days after TPA
5. Sixty day after TPA
6. Thirty days after stop TPA

Example VI

Case Studies

Results of treatment of initially symptomatic AIDS patients treated with TPA according to the protocols of Example III, IV, and V. Patients who participated in multiple studies are in some cases identified by more than one patient number. All patient identification numbers correspond to the patient numbers in Tables 1-7.

Patient #1 and 15:

H.L.Y., female, 35, participated in all three clinical studies, diagnosed with AIDS and had clear symptoms of this disease in 2003. At the time the first study began, she had frequent fever, diarrhea, oral lesions, poor appetite, weight loss, left eye vision loss (syncytia formation) and coughing (tuberculosis). The patient started to receive antiviral medications Stavudine (D_4T), Lamivudine (3TC), Nevirapine (NVP) and Zidovudine (AZT) in 2004. Despite anti-AIDS drugs, she had a CD4 count of 3 and was unable to perform any physical work.

During the first study following the protocol of Example III, above, she experienced an increase in body temperature of 38-39° C. on four different occasions that lasted 2 to 4 hours. After treatment with TPA, there was a gradual improvement in symptoms. Her appetite improved and diarrhea, oral lesions, and fatigue disappeared but her eyesight remained impaired. She gained some weight and reported being able to resume housework. She continues to receive antiviral therapy. There appears to be no correlation in improvements in symptoms and changes in her CD 3, 4, 8 levels and viral count.

H. L. Y. participated in the second study described in Example IV, above. At the initiation of the second study she has no symptoms of AIDS. During this subsequent treatment with TPA she experienced no adverse effects. After both the first and third cycle of treatment with TPA, her CD3, CD4,

and CD8 levels increased as did her white blood cell count. Her HIV count was somewhat higher, but she is able to function normally and continues to have no symptoms of AIDS.

H.L.Y. participated in the third study described in Example V, above. At the initiation of the third study, she was still having problems with her eye. During the third study, she experienced a fever of 38-38.5° C. during the third and fourth day of TPA infusion. No AIDS symptoms returned during either the study or the 60 day observation period. Except for her sight, she remains symptom free, feels normal and is able to conduct normal activities. She reinitiated antiviral therapy after completion of the 60 day observation period and remains under the care of a physician.

Patient #2:

C.X., female, 49, participated in first clinical study, diagnosed with AIDS and had clear symptoms of this disease in 2004. She had mild oral lesions, fatigue, skin thrush, fever and poor appetite. Some of these symptoms were due to herpes virus. She had been treated with AZT, DDI and NVP but drug treatment was terminated due to side effects. She received no drugs for 3 months prior to TPA treatment. She was hospitalized frequently and was unable to work. Her CD4 count prior to treatment was 26.

During TPA treatment according to the protocol of Example III, she experienced an increase in body temperature of 37.5 to 38 degrees centigrade on three different occasions that lasted 1-2 hours. After treatment with TPA, her oral lesions, skin thrush and fever disappeared. Her appetite improved sufficiently so that she gained weight and had sufficient energy to resume housework. She remained symptom free for five months and was not given any anti-AIDS drugs during this period. There appeared to be no correlation between the improvement in symptoms and her CD 3, 4, 8 levels and viral count.

Patient #2a

M. S., male, 48, participated only in the second clinical study, had frequent fever, diarrhea, weight loss, a weak immune system, severe depression and was unable to work.

During treatment with TPA according to the protocols of Example IV, his body temperature increased to 38.5 to 39 degrees centigrade on five occasions for 2 to 4 hours.

After the third cycle of TPA treatment, the fever and diarrhea were no longer a problem. His CD3, CD4 and CD8 counts trended upwards as did the WBC and HIV count. His physical and mental condition returned to normal and he is able to work.

Patient #3:

Y.P., male, 51, participated only in the first clinical study, diagnosed with AIDS and had clear symptoms of this disease in 2004. His major symptoms were diarrhea, fatigue, weight loss, anemia and purple marks on the skin of both legs; and he could only do light work. He was being treated with AZT, DDI and NVP but a serious anemia resulted in the termination of drug treatment four months prior to being given TPA. His initial CD4 count was 32.

During TPA treatment according to the protocol described in Example III, he experienced an increase in body temperature of 38 to 39° C. on three occasions that lasted 1 to 2 hours. After treatment with TPA, there was a marked improvement in his symptoms and he was able to return to work involving heavy labor and is leading a normal life. He was symptom free for five months after TPA therapy and was not treated with antiviral drugs during this period. There appeared to be no correlation between CD 3, 4, and 8 levels and improvement in symptoms but there was some increase in viral count.

Patient #4:

L.W., male, 34, participated in only the first clinical study, tested positive for HIV and had clear symptoms of this disease in 2004. His major symptoms were diarrhea, fever, weight loss, cough (tuberculosis), right side neck lymph node enlargement and he was unable to work. His initial response to treatment was poor. The schedule of antiviral medication of 3TC, DDI and NVP was irregular and was stopped during TPA therapy. His initial CD4 count was 173.

During treatment with TPA according to the protocol of Example III, he experienced an increase in body temperature of 38 to 39° C. on five occasions that lasted 0.5 to 1 hours. After treatment, the occasional bout of diarrhea was treated successfully with and an anti-diarrhea drug. An improvement in appetite has resulted in an increase in weight and energy that resulted in his returning to a regular work schedule. The lymph node returned to normal size. He continues to be treated with anti-viral drugs. There appeared to be no correlation between the improvements in symptoms, CD3, 4, 8 levels and viral count.

Patient #5 and 3a:

H.S., female, 37, participated in the first two clinical studies, tested positive for HIV and had clear symptoms of the disease in of 2004. At the time the first study began, her major symptoms were skin thrush, hair loss, mouth infection, weight loss and fatigue. She was being treated with D_4T, DDI, and NVP but treatment was stopped due to loss of kidney function. She had an initial CD4 count of 106 but could handle regular labor work.

During treatment with TPA according to the protocol of Example III, she experienced in increase in body temperature of 37.5 to 38° C. on five occasions that lasted 0.5 to 1.0 hours. After treatment with TPA, no improvement in symptoms occurred. Treatment with anti-viral drugs was resumed without return of the previous side effects and the intensity of her symptoms were reduced after one month. This treatment is being continued and she has returned to work. There appeared to be no correlation between the improvement in symptoms and changes in the CD 3, 4, and 8 levels or the HIV count.

At the time of the second study, she had no symptoms of AIDS and suffered no adverse effects to the course of treatment described in Example IV. After the second study, her CD3, CD4 and CD8 levels trended upwards as did her white blood count and platelet levels. Her HIV count was initially undetectable, but increased after the third cycle of treatment. She is currently able to work.

Patient #6, #4a, and #17:

H.S.C., male, 36, participated in all three clinical studies, tested positive for HIV and had clear but mild symptoms in 2004. At the time the first study began, he suffered from dizziness, headache, poor appetite and an increased susceptibility to upper respiratory tract infections but was able to work regularly as a laborer. He was being treated with antiviral drugs AZT, DDI and NVP but terminated their use due to adverse reactions. His initial CD4 level was 232.

During treatment with TPA according to the protocol of Example III, he did not experience an increase in body temperature or any other side effect. After treatment, his symptoms remained unchanged and a reduction in platelets appeared unrelated to TPA treatment. He continued to be treated with antiviral drugs and is able to work as before. There appeared to be no correlation between the improvement in symptoms and the CD 3, 4, and 8 levels and viral load.

At the time of the second study, he had no symptoms and his immune system appeared to be functioning normally. During the second study according to Example IV, he again suffered no side effects from treatment with TPA. His CD3,

CD4, and CD8 count increased somewhat as did his white blood cell count. The viral load was initially undetectable but increased after the third cycle of treatment. However, he does not have any symptoms of AIDS and has returned to work.

At the initiation of the third clinical study, he had no symptoms. During treatment with TPA according to the protocol of Example V, he experienced an incident of local irritation due to a leaking needle on day 32 but was treated successfully in three days. He remains symptom free, feels normal, and is able to do heavy labor. He started antiviral therapy after completion fo the 60 day observation periods and remains under the care of a physician.

Patient #7, #5a and #16:

H. C. L., male, 49, participated in all three clinical studies, tested positive for HIV and had clear symptoms of the disease in 2004. His major symptoms at the time of the first study were weight loss, skin thrush, fatigue, poor appetite and coughing (tuberculosis) but he was able to do light work. He was treated simultaneously with D_4T, DDI, NVP and antituberculosis medication. His initial CD4 count was 10.

During treatment with TPA according to the protocol outlined in Example III, he experienced an increase in body temperature to 38° C. on two occasions accompanied by mild dizziness and headache. After treatment, his symptoms remained unchanged and antiviral therapy was resumed one month later. With time, his cough, appetite and energy level improved and he is able to work. He continued both antiviral and anti-tuberculosis medication. There appeared to be no correlation between improvements in symptoms and his CD3, 4, and 8 levels or viral load.

At the time of the second clinical investigation, he had no symptoms of AIDS and his immune system appeared to be functioning normally. He suffered no adverse effects from treatment TPA during the second clinical investigation. After treatment, his CD4 level was unchanged, but his CD3 and CD8 levels trended upwards as did his white blood cell count. His viral load was undetectable. He has not had any symptoms of AIDS and has returned to work.

At the start of the third clinical investigation, he was not experiencing AIDS symptoms. During treatment according to the protocol outlined in Example V, he suffered from a fever on one occasion. He remains symptom free, feels normal, and is able to do heavy labor. He re-started antiviral drugs after completion of the 60 day observation period and remains under the care of a physician.

Patient #8, #6a, and 18:

Y.X.O., female, 36, participated in all three clinical studies, tested positive for HIV in 2004. Her major symptom at the time of the first study was an increased susceptibility to upper respiratory tract infection. She was treated with AZT, DDI and NVP. At the start of the study, her CD4 level was 524 and she could handle regular labor work.

During treatment with TPA according to the protocol of Example III, she experienced an increase in body temperature to 38.5° C. on one occasion that lasted four hours. After treatment, the frequency of her colds decreased and she had no other symptoms. She continued to be treated with antiviral drugs and is able to work. There appeared to be no correlation between the improvement in symptoms and her CD 3, 4, or 8 levels or viral load.

At the time of the second clinical investigation, she had no symptoms of AIDS and her immune system appeared to be functioning normally. During the second study, according to the protocols of Example IV, her body temperature again rose to 38.5 degrees centigrade for two hours on a single occasion. After treatment, her CD3 and CD8 levels increased somewhat while her CD4 and white blood cell count remained unchanged. Her viral load is undetectable. She appears normal and is able to work at physically demanding tasks.

At the time of the third clinical investigation she was symptom free. The only side effects from treatment according to the protocol of Example V was as fever of 38-39° C. on the second day of the treatment that lasted for two hours and skin irritation from a leaking needle on day 36 that cleared in two days. She remains symptom free, feels normal and is able to do heavy labor. She re-started antiviral therapy after completion of the 60 day observation period and remains under the care of a physician.

Patient #9 and #7a:

C.T.F., male, 44, participated in the first two clinical studies, tested positive for HIV and had clear symptoms of the disease in 2004. His symptoms at the initiation of the first study included persistent diarrhea, dizziness, headaches, poor appetite, weight loss and fatigue. He had a positive response to AZT, DDI and NVP treatment and blood HIV count was near the lowest limit. Despite the positive response, his symptoms persisted and he checked into the hospital due to diarrhea that persisted for 20 days. He was very depressed and unable to do any work.

During treatment with TPA according to the protocol of Example III, he experienced an increase in body temperature of 37.5 to 38° C. on six occasions that lasted 2 to 4 hours. A leaking needle caused a serious skin irritation during one administration of TPA but was treated successfully. After eight treatments with TPA, the mild dizziness and headache persisted but the incidence of diarrhea began to decrease and his appetite improved. A week later, his diarrhea was completely gone and he had a normal appetite. He was able to return to work and is receiving antiviral drug therapy. There appeared to be an upward trend of CD3, 4, 8 levels and the HIV count was undetectable.

At the time of the second clinical investigation, he had no symptoms of AIDS and his immune system appeared to be functioning normally. During TPA treatment according to the protocol of Example IV, he suffered no adverse effects. After treatment, his CD3, CD4 and CD8 levels increased somewhat while his white blood cell count remained unchanged. His HIV count continues to be undetectable. He is able to do strenuous work.

Patient #10 and #8a:

W.F.W., Female, 47, participated in the first two studies, tested positive for HIV and had clear symptoms of the disease in 2003. Her symptoms at the initiation of the first study included low body temperature, diarrhea, low platelet count, coughing blood, bloody bowel movements, dizziness, headache, poor appetite, weight loss, fatigue with mild skin thrush and deep depression. She was hospitalized on one occasion for two months because of bloody bowel movements. She was very depressed and unable to work. She did not respond positively to the AZT, DDI and NVP treatment and her symptoms were not under control.

During her first treatment with TPA according to the protocol of Example III, she experienced an increase in body temperature to 38.5° C. on one occasion that lasted 4 hours. After TPA treatment, her dizziness, headache and diarrhea gradually lessened. Eventually, her appetite led to a weight gain and an improvement in her energy level. Her platelet count rose from 30,000 to 110,000 per microliter and the skin thrush and diarrhea were eliminated. She was able to work again and was treated with antiviral drugs. She had fever and diarrhea occasionally that she was able to control with drugs.

Six months later she suffered from mild headaches and dizziness and underwent a second treatment with TPA. During her second treatment with TPA, she experienced an

increase in body temperature to 37.5 to 38° C. on five occasions that lasted 2 to 4 hours. Twenty hours after the 13th injection of TPA, her temperature reached 40.5 degrees centigrade and lasted for several hours. It was concluded that the increase in temperature was not related to TPA therapy.

After her second treatment with TPA, her symptoms disappeared, her appetite improved and she gained weight, which enabled her to regain her energy, return to work and lead a normal life. She was free of symptoms for one year and has had few colds in the first six months after the second TPA treatment. There appears to be an upward trend for the CD 3, 4, and 8 levels and the HIV counts.

At the time of the second clinical trial according to the protocol of Example IV, this patient continued to display no symptoms of AIDS and her immune system appeared to be functioning normally. She suffered no adverse effects during treatment. After treatment, her CD3, CD4 and CD8 counts increased somewhat as did her WBC. Her HIV count increased somewhat. Since the studies, she has been healthy and engaged in laborious work.

Patient #11 and 9a:

C.T.L., female, 40, participated in the first two studies, was diagnosed with AIDS and had clear symptoms of this disease in 2003. At the initiation of the first study she had persistent diarrhea, low body temperature, oral lesions, severe skin thrush, itching, purple blotches on her face and lips, dizziness, headache, poor appetite, and fatigue and depression. She responded poorly to AZT, 3TC and NVP treatment. Her symptoms were not under control and she was unable to work. Her initial CD4 count was 40.

During her first treatment with TPA, she experienced an increase in body temperature to 38 to 39° C. on four occasions that lasted 2 to 4 hours. She had shortness of breath on two occasions that lasted 20 to 30 minutes each.

After the sixth dose of TPA, her skin thrush began to disappear and upon completion of TPA treatment, the dizziness, headache, fever and skin thrush were improving and gradually faded away. Her appetite, physical condition and depression improved sufficiently for her to return to work.

This patient had a second treatment with TPA 18 months later due to the return of symptoms including mild skin thrush, diarrhea and dizziness. During this second treatment, she experienced an increase in body temperature to 37.5 to 38° C. three times that lasted 2 to 4 hours. There were no other adverse reactions. After treatment with TPA, her symptoms disappeared completely and her physical condition improved sufficiently to allow her to return to work. She has been without symptoms for one year and she has rarely had a cold. There appears to be an upward trend in CD 3, 4, and 8 levels, but her HIV counts did not change.

At the time of the second clinical study according to the protocol of Example IV, this patient exhibited symptoms of AIDS including headache, dizziness, poor appetite and a weak immune function. She suffered no adverse effects during treatment. After treatment, her CD3 and CD8 levels increased while her CD4 count was unchanged. Her HIV count increased slightly but no other changes were observed. Her mental and physical condition has improved considerably and she is doing strenuous physical work.

Patient #12 and #10a:

C.C.L., female, 39, participated in the first two studies, diagnosed with AIDS and had clear symptoms of this disease in 2003. At the initiation of the first study she had persistent low body temperature, skin thrush, dizziness, headache, poor appetite, oral lesions, fatigue and deep depression. She was treated with AZT, 3TC and NVP but had poor results and she was unable to work. Her initial CD4 count was 84.

This patient was treated with TPA twice during the period March 2005 to March 2006. During the first treatment with TPA, she experienced an increase in body temperature to 38 to 38.5° C. on eight occasions that lasted 2 to 4 hours. She experienced shortness of breath on one occasion for 15 minutes and suffered a skin irritation due to a leaking needle.

After the seventh injection, her oral lesions disappeared. Upon completion of all the injections, all symptoms disappeared and her physical condition improved sufficiently for her to return to work.

Six months later, this patient was re-retreated with TPA due to the return of light diarrhea and dizziness. She experienced an increase in body temperature to 37.5 to 38° C. centigrade on six occasions associated with TPA administration that lasted 2 to 6 hours. Starting with the eighth injection, the dose was increased from approximately 150 μg to 250 μg TPA. No adverse effects occurred. Upon completion of TPA therapy, all her symptoms disappeared. Her physical condition was restored to normal and she returned to work and has had a normal life. She has been symptom free for one year and has rarely had a cold. There were no changes in CD 3, 4, or 8 levels, but her HIV count increased.

At the time of the second clinical study, this patient had no symptoms of AIDS though she did have a weakened immune system. She was treated according to the protocol of Example IV and suffered no adverse effects. After treatment, there were slight increases in her CD3, CD4 and CD8, and modest increases in WBC, RBC and HGB while platelets appeared to decrease. The HIV count was reduced somewhat. She has been healthy and engaged in strenuous physical work since her treatments.

Patient #13:

L.F.L., female, 53, diagnosed with AIDS in 2004, participated in only the third clinical study. She presented with mild symptoms of poor appetite and weight loss. Long term antiviral drugs were effective and caused her virus count to decrease below detectable levels and CD3, CD4 and CD8 counts to increase to a high level. She had no symptoms prior to TPA treatment and had no side effects from its administration. She remains symptom free, feels normal, and is able to conduct normal activities. She re-started antiviral drug therapy after completion fo the 60 day observation period.

Patent #14:

K.S.M., female, 45, diagnosed with AIDS in 2004, participated in only the third clinical study. Her symptoms were mild and consisted of poor appetite and frequent colds. She had been treated with antiviral drugs, but stopped due to severe liver toxicity. She had no symptoms prior TPA treatment and the only TPA side effect was irritation due to a leaking needle on day 43 that was easily treated. No AIDS symptoms occurred during the entire treatment and observation period. She feels normal and is able to conduct her usual activities. After completion of the 60 day observation period she was lost to the study and did not renew antiviral therapy.

Example VII

Treatment of Relapsed/Refractory Malignancies with TPA

Patients with histologically documented relapsed/refractory hematologic malignancy/bone marrow disorders are treated with a combination of TPA (Xichuan Pharmaceuticals, Nan Yang, Henan, China), dexamethasone and choline magnesium trisalicylate. Comparable methods as set forth below for demonstrating the therapeutic use of TPA in the treatment of Acute Myelogenous Leukemia (AML) will be

applied to demonstrate the use of TPA for treating other neoplastic conditions and malignancies. Other neoplastic conditions and malignant disorders amenable to treatment using the methods and compositions of the invention include various forms of cancer, including blood and bone malignancies and solid tumors of various types. In addition to the specific protocols herein, successful treatment and/or remission will be determined for different targeted neoplastic and malignant conditions using any of a wide variety of well known cancer detection and assessment methods—for example by determining size reduction of solid tumors, histopathological studies to evaluate tumor growth, stage, metastatic potential, presence/expression levels of histological cancer markers, etc.

AML is an aggressive disease that generally warrants urgent and intensive therapy. The average patient age at AML diagnosis is 64-68 years old, and patients over the age of 60 treated with standard chemotherapy are cured of their disease<20% of the time. Patients who develop AML after an antecedent hematologic disorder or prior leukemogenic chemotherapy/radiation therapy have similarly poor outcomes, as do patients whose disease is associated with specific adverse cytogenetic and clinical features. Hence, most patients diagnosed with AML have patient and/or disease-related features that are associated with a very poor prognosis. For patients with relapsed disease, no standard non-transplant therapy has demonstrated the capacity for cure. For these patients, AML is often a fatal disease. New approaches to the therapy of AML are needed.

Employing the methods and compositions of the instant invention, TPA, is developed as a therapeutic agent for treating patients with AML, based on TPA's novel role in modulating intracellular signaling pathways, it's capacity to induce differentiation and/or apoptosis in cell lines, and clinical data indicating the effectiveness of TPA in treating neoplastic and malignant disorders, including myeloid malignancies.

Thus far clinical evaluation of TPA has demonstrated that TPA exerts direct therapeutic cytotoxic effects in at least a subset of AML cases, as measured by cell viability and apoptosis assays. In all primary cultures analyzed by Western analysis, TPA strongly induced ERK phosphorylation by 1 hour in culture. TPA's cytotoxic effect on primary AML cells is associated with the subsequent loss of the phospho-ERK pro-survival signal after 24 hour ex vivo exposure. This observation is in good agreement with other studies that reported decreased primary AML survival after pharmacological interruption of ERK signaling by MEK inhibitors, such as PD98059, U0126 and PD 184352. In our studies, loss of ERK signaling was associated with induction of ERK phosphatases.

In addition to protein kinase C and ERK activation, TPA is a known inducer of NF-κB, a pro-survival transcription factor often constitutively active in AML blasts and leukemic stem cells. Recent work from our laboratory has demonstrated that AML cell NF-κB can be inhibited in vivo with 48 h of treatment with dexamethasone+choline magnesium trisalicylate (CMT). In addition, we have shown that dexamethasone can induce MKP-1 ERK phosphatase expression and enhance TPA cytotoxicity on primary AML samples. In this context, we have chosen in exemplary embodiments below to use dexamethasone and CMT as adjunctive medications to be used 24 h pre- and 24 h post treatment with TPA. These medications are well-tolerated and anticipated to reduce inflammatory adverse effects of treatment and enhance TPA cytotoxicity by increasing ERK phosphatase expression and inhibiting NF-κB. In addition dexamethasone and CMT will be used as adjunctive medications because they are anti-inflammatory, may ameliorate adverse effects, and may enhance anti-leukemic activity by inhibition of the anti-apoptotic effects of constitutive NF-κB expression and induction of phosphatases that decrease signaling pathway activity.

An initial TPA Phase 1 study enrolled 35 patients [23 with relapsed/refractory AML, 2 with other myeloid malignancies (CML-blast crisis, myelodysplasia with excess blasts), 3 with Hodgkin's Disease, 3 with non-Hodgkin's lymphoma and 4 with solid tumors]. The majority of patients had relapsed/refractory AML. Our clinical results include one AML patient with stable disease for >5 months, who received 8 TPA infusions. In a second AML patient, a pronounced (5-fold) decline in the number of circulating blasts was seen following TPA administration. This decline in leukemic blasts persisted for 4 weeks, and the patient eventually died from a fungal infection. Finally, a patient with relapsed and refractory Hodgkin's disease despite high dose chemotherapy with autologous stem cell rescue had a partial remission of a chest wall mass after TPA administration. TPA dose escalation has been completed, in the last cohort 2 out of 3 patients treated at a dose of 0.188 mg/m2 d1-5, 8-12 experienced grade III non-hematologic dose limiting toxicities (DLT), establishing the maximum tolerated TPA dose as a single agent at 0.125 mg/m2/d on d1-5 and 8-12.

In the case of AML and other hematologic malignancies, patients are given an initial dose of TPA of 1 mg/week×3 weeks (days 1, 8, 15) administered with continuous/intermittent pulse oximetry for 6 hours. Twenty four hours prior to initiation of TPA therapy, patients are given 10 mg of dexamethasone every six hours and 1500 mg of choline magnesium trisalicylate (CMT) every eight hours continuing until 24 hours after administration of TPA. After administration of the initial dose of TPA, patients have a two week rest period after which they may be reevaluated. Those patients that have a disease response or stabilization from the initial dose of TPA are treated for up to six cycles of twenty-eight days according to the protocol below.

Following the two week rest period, patients are pre-medicated with Tylenol 650 mg and Benadryl 25-50 mg (depending on the patient's size and age) thirty minutes prior to administration of TPA. They are then given an intravenous infusion of TPA through a central venous catheter daily for 5 days a week for two consecutive weeks followed by a 2-week rest period. TPA is administered at a dose of 1 mg in 200 ml of normal saline over 1 hour. Twenty four hours prior to initiation of TPA therapy, patients are given 10 mg of dexamethasone every six hours and 1500 mg of choline magnesium trisalicylate continuing every eight hours until 24 hours after administration of the TPA.

Blood levels of TPA are measured prior to and after infusion using a bioassay that measures organic solvent extractable differentiation activity. 1 ml of blood is extracted twice with 5 ml of ethyl acetate, redissolving the extraction residue in 50 μL of ethanol and addition of an aliquot of HL60 cells. After 48 hours, adherent cells are measured.

Tests are also run on blood samples taken prior to and after infusion with TPA to determine levels of white blood cells, platelets, and neutrophils. The samples are additionally analyzed for the presence of myeloblasts and Auer rods. These and continuing experiments will further elucidate the therapeutic cytotoxic and other effects that TPA elicits against neoplastic cells in AML and other neoplastic and malignant conditions.

Example VIII

Measurement of the Modulation of ERK Activation

Phospho-ERK levels are measured in circulating malignant cells in patients with leukemia and in peripheral blood

mononuclear cells in lymphoma/solid tumor patients. A blood sample is taken from patients treated according to the protocol of Example VII both prior to and after administration of TPA.

In leukemia patients with a WBC≥1000 per μL, flow cytometry is performed on a blood sample using cell surface antigen-specific and phospho-ERK specific antibodies directly conjugated to fluorophores (BD Biosciences, San Jose, Calif.). Samples are taken pre-administration of TPA and one our after infusion of TPA on days 1, 2, and 11 in the initial treatment according to the protocol of Example VII and days 1 and 11 in subsequent cycles. In leukemia patients with an absolute leukemic blast number≥2500 per μL and other non-leukemic patients, peripheral blood samples are taken on days 1, 8 and 15 of the initial cycle according to the protocol of Example VII prior to and 1 and 4 hours post infusion. Samples are also analyzed using Western blot analysis for phosphor-ERK, and total ERK1/2 levels to confirm the results obtained from the flow cytometry and correlated to clinical responses.

The foregoing analyses will further elucidate TPA's role in treatment of neoplastic and malignant conditions, including TPA's cytotoxic effect on malignant cells, exemplified by primary AML cells, and the associated reduction by TPA of the phosphor-ERK pro-survival signal.

Example IX

Measurement of NF-κB modulation

In prior studies we have shown that NF-κB activity can be modulated in patients following administration of TPA with dexamethasone. Additionally, dexamethasone has been shown to induce MKP-1 ERK phosphatase expression and enhance TPA cytotoxicity. The following studies are designed to further elucidate how NF-κB activity is therapeutically modulated in patients treated with TPA plus dexamethasone.

NF-κB binding is measured in patient peripheral blood samples at baseline and pre and post infusion from patients treated with TPA according to Example VII using ELISA-based assays (BD Bioscience, San Jose, USA). NF-κB levels are quantified using chemiluminescent intensity to detect binging in limiting amounts of cellular extract using a 96-well format. Additionally, electrophoretic mobility shift assays are performed to measure NF-κB binding in peripheral blood samples from leukemia patient with an absolute leukemic blast number≥2500 per μL and other non-leukemic patients with normal white blood cell counts.

The foregoing studies will further PA is an inducer of NF-κB, however these experiments demonstrate that AML cell NF-κB can be inhibited with treatment with dexamethasone and choline magnesium trisalicylate.

Example X

Determination of changes in Leukemic Gene Expression

TPA induces RNA levels of several dual specificity phosphatases capable of terminating pro-survival ERK pathway signaling. A blood sample taken pre and post infusion from patients with AML treated with TPA according to Example VII is used to study RNA expression of AML signaling components such as the MAPK-specific DUSPs using quantitative realtime RT-PCR and oligonucleotide microarray analysis.

Although the foregoing invention has been described in detail by way of example for purposes of clarity and understanding, it will be apparent to the artisan that certain changes and modifications may be practiced within the scope of the appended claims which are presented by way of illustration not limitation. In this context, various publications and other references have been cited with the foregoing disclosure for economy of description. Each of these references is incorporated herein by reference in its entirety for all purposes. It is noted, however, that the various publications discussed herein are incorporated solely for their disclosure prior to the filing date of the present application, and the inventors reserve the right to antedate such disclosure by virtue of prior invention.

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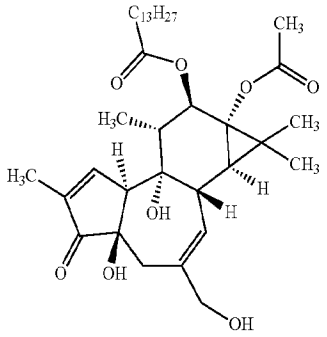
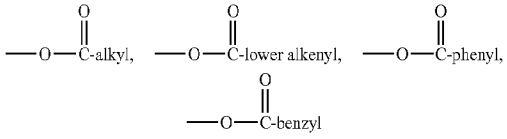
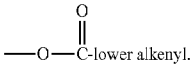
We claim:

1. A method for treating HIV infection or disease-in a mammalian subject comprising administering an effective amount of a phorbol ester of Formula II, a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, or polymorph thereof to said mammalian subject

Formula II







2. The method of claim **1**, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

3. The method of claim **1**, further comprising administering at least one secondary or anti-retroviral or other adjunctive therapeutic agent with said phorbol ester.

4. The method of claim **3**, wherein the at least one secondary anti-retroviral or other adjunctive therapeutic agent is administered to said subject simultaneously with, prior to, or after, administration of said phorbol ester.

5. The method of claim **3**, wherein the at least one secondary anti-retroviral or other adjunctive therapeutic agent is selected from the group consisting oft protease inhibitors, nucleoside reverse transcriptase, non-nucleoside reverse transcriptase inhibitors, combination drugs, entry and fusion inhibitors, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, paclitaxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, valganciclovir; integrase inhibitors, microbicides, and IL-2.

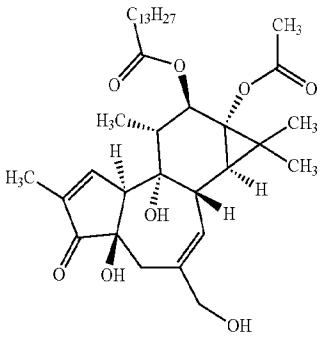
6. The method of claim **1**, wherein said effective amount is between about 10 and 1500 μg of said phorbol ester every other day.

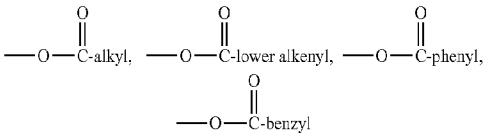
7. The method of claim **1**, wherein said effective amount is between about 150 to 500 μg of said phorbol ester every other day.

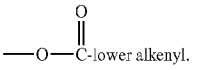
8. The method of claim **1**, wherein said effective amount of said phorbol ester is administered once per day.

9. A method for treating one or more symptoms or conditions of HIV infection or AIDS in a mammalian subject comprising administering an effective amount of phorbol ester of Formula II, a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, or polymorph thereof to said mammalian subject

Formula II







R is selected from hydrogen, and substituted derivatives thereof.

10. The method of claim **9**, wherein the phorbol ester is 12-O-tetradecanoylphobol-13-acetate.

11. The method of claim **9**, further comprising administering at least one secondary anti-retroviral or other adjunctive therapeutic agent with said phorbol ester.

12. The method of claim **11**, wherein the at least one secondary anti-retroviral or other adjunctive therapeutic agent is administered simultaneously with, prior to, or after, administration of said phorbol ester.

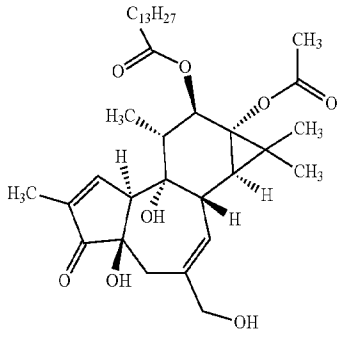
13. The method of claim **11**, wherein the at least one secondary anti-retroviral or other adjunctive therapeutic agent is selected from the group consisting oft protease inhibitors, nucleoside reverse transcriptase, non-nucleoside reverse transcriptase inhibitors, combination drugs, entry and fusion inhibitors, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, pacli-

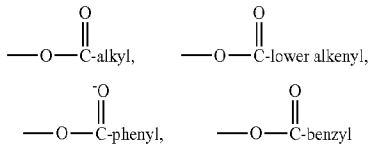
taxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, valganciclovir; integrase inhibitors, microbicides, and IL-2.

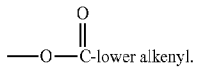
14. The method of claim **9**, wherein the one or more symptoms is selected from the group consisting of oral lesions, fatigue, skin thrush, fever, lack of appetite, diarrhea, apthous ulcers, malabsorbtion, thrombocytopenia, weight loss, anemia, and lymph node enlargement, *mycobacterium avium* complex, salmonellosis, syphilis, neuroshyphilis, turberculosis, bacillary angiomatosis, aspergillosis, candidiasis, coccidioidomycosis, listeriosis, pelvic inflammatory disease, Burkitt's lymphoma, cryptococcal meningitis, histoplasmosis, Kaposi's sarcoma, lymphoma, systemic non-Hodgkin's lymphoma, primary CNS lymphoma, cryptosporidiosis, isosporiasis, microsporidiosis, *pneumocystis carinii* pneumonia, toxoplasmosis, cytomegalovirus, hepatitis, herpes simplex, herpes zoster, human papiloma virus, molluscum contagiosum, oral hairy leukoplakia, and progressive multifocal leukoencephalopathy.

15. A method for treating HIV infection in a mammalian subject with AIDS comprising administering an effective amount of a phorbol ester of Formula II, a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, or polymorph thereof, to said mammalian subject

Formula II



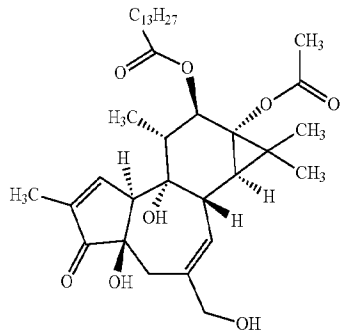


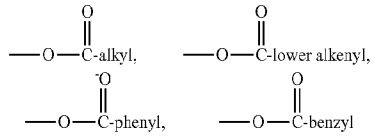


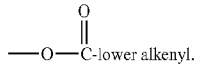
16. The method of claim **15**, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

17. A method for activating latent reservoirs of HIV comprising administering an effective amount of a phorbol ester of Formula II, a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, or polymorph thereof to said mammalian subject

Formula II







18. The method of claim **17**, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

19. The method of claim **17**, further comprising administering a secondary anti-retroviral or other adjunctive therapeutic agent with said phorbol ester.

20. The method of claim **19**, wherein the secondary anti-retroviral or adjunctive therapeutic agent is administered to said subject simultaneously with, prior to, or after, administration of said phorbol ester.

21. The method of claim **19**, wherein the secondary anti-retroviral or adjunctive therapeutic agent is selected from the group consisting of protease inhibitors, nucleoside reverse transcriptase, non-nucleoside reverse transcriptase inhibitors, combination drugs, entry and fusion inhibitors, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, paclitaxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, valganciclovir; integrase inhibitors, microbicides, and IL-2.

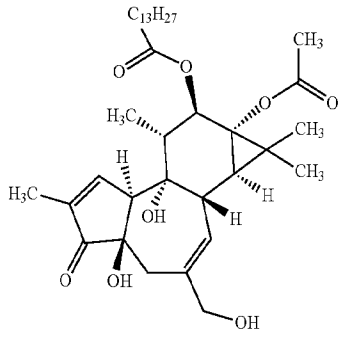
22. The method of claim **17**, wherein said effective amount is between about 10 and 1500 μg of said phorbol ester every other day.

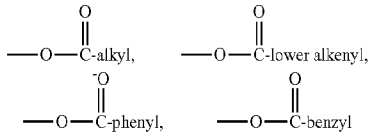
23. The method of claim **17**, wherein said effective amount is between about 150 to 500 μg of said phorbol ester every other day.

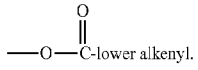
24. The method of claim **17**, wherein said effective amount of said phorbol ester is administered once per day.

25. A method of increasing the expression of Th1 cytokines comprising administering an effective amount of a phorbol ester of Formula II, a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, or polymorph thereof to said mammalian subject

Formula II







26. The method of claim 25, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

27. The method of claim 25, further comprising administering a secondary or other adjunctive therapeutic agent with said phorbol ester.

28. The method of claim 27, wherein the secondary or adjunctive therapeutic agent is administered to said subject simultaneously with, prior to, or after, administration of said phorbol ester.

29. The method of claim 27, wherein the secondary or adjunctive therapeutic agent is selected from the group consisting oft protease inhibitors, nucleoside reverse transcriptase, non-nucleoside reverse transcriptase inhibitors, combination drugs, entry and fusion inhibitors, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, paclitaxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, valganciclovir; integrase inhibitors, microbicides, and IL-2.

30. The method of claim 25, wherein said effective amount is between about 10 and 1500 μg of said phorbol ester every other day.

31. The method of claim 25, wherein said effective amount is between about 150 to 500 μg of said phorbol ester every other day.

32. The method of claim 25, wherein said effective amount of said phorbol ester is administered once per day.

* * * * *

US 20140017194A1

(19) United States

(12) Patent Application Publication

(10) Pub. No.: US 2014/0017194 A1

(43) Pub. Date: Jan. 16, 2014

Han et al.

(54) **COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS**

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(21) Appl. No.: **13/794,467**

(22) Filed: **Mar. 11, 2013**

Related U.S. Application Data

(63) Continuation of application No. 13/595,072, filed on Aug. 27, 2012, now abandoned, which is a continuation of application No. 12/023,753, filed on Jan. 31, 2008, now abandoned.

(60) Provisional application No. 60/898,810, filed on Jan. 31, 2007.

Publication Classification

(51) **Int. Cl.**

A61K 31/225	(2006.01)
A61K 31/573	(2006.01)
A61K 45/06	(2006.01)
A61K 31/606	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 31/225*** (2013.01); ***A61K 31/606*** (2013.01); ***A61K 31/573*** (2013.01); ***A61K 45/06*** (2013.01)

USPC **424/85.2**; 424/85.7; 424/130.1; 424/94.5; 424/94.6; 514/43; 514/100; 514/163; 514/171; 514/548

(57) **ABSTRACT**

Methods and compositions containing a phorbol ester or a derivative of a phorbol ester are provided for the treatment of cytopathic diseases. Cytopathic diseases may be caused by a variety means such as viral infections like HIV and AIDS, or the development of neoplasms in a mammalian subject. The methods and compositions of the invention are effective for inhibiting de novo HIV infection, upregulating viral expression from latent provirus, inhibiting HIV-induced cytopathic effects, down regulating the HIV receptor, increasing Th1 cytokine expression, decreasing Th2 cytokine expression, increasing ERK phosphorylation, inducing apoptosis in malignant cells, inducing remission, maintaining remission, as chemotherapeutic agents, as well as for decreasing symptoms of cytopathic diseases and opportunistic infections that may accompany such diseases. Additional compositions and methods are provided which employ a phorbol ester or derivative compound in combination with at least one additional agent such as those used in HAART protocols, therapeutic agents used to treat opportunistic infections due to HIV, or chemotherapeutic agents to yield more effective treatment tools against cytopathic diseases in mammalian subjects.

COMPOSITIONS AND METHODS OF USE OF PHORBOL ESTERS

RELATED APPLICATIONS

[0001] This application claims priority benefits of U.S. Provisional patent application Ser. No. 60/898,810, filed Jan. 31, 2007, which is incorporated herein in its entirety by reference.

TECHNICAL FIELD

[0002] The present invention relates generally to the field of cytopathic diseases. More specifically, the invention relates to compositions containing and methods of using phorbol esters to treat cytopathic conditions and diseases that cause such cytopathic conditions.

BACKGROUND

[0003] Phorbol is a natural, plant-derived organic compound of the tigliane family of diterpenes. It was first isolated in 1934 as a hydrolysis product of *croton* oil derived from the seeds of *Croton tiglium*, a leafy shrub of the Euphorbiaceae family that is native to Southeastern Asia. Various esters of phorbol have important biological properties including the reported ability to mimic diacylglycerols and activate protein kinase C (PKC), modulating downstream cell signaling pathways including the mitogen-activated protein kinase (MAPK) pathways. Phorbol esters are additionally thought to bind to chimaerins, the Ras activator RasGRP, and the vesicle-priming protein Munc-13 (Brose N, Rosenmund C., JCell Sci; 115:4399-411 (2002)). Some phorbol esters also induce nuclear factor-kappa B (NF-κB). The most notable physiological property of phorbol esters is their reported capacity to act as tumor promoters.

[0004] 12-O-tetradecanoylphorbol-13-acetate (TPA), also called phorbol-12-myristate-13-acetate (PMA), is a phorbol ester used in models of carcinogenesis as an inducer for differentiation and/or apoptosis in multiple cell lines and primary cells. TPA has also been reported to cause an increase in circulating white blood cells and neutrophils in patients whose bone marrow function has been depressed by chemotherapy. (Han Z. T. et al. Proc. Natl. Acad. Sci. 95, 5363-5365 (1998)) and inhibit the HIV-cytopathic effects on MT-4 cells. (Mekkawy S. et al., Phytochemistry 53, 47-464 (2000)). However, due to a variety of factors, including caustic reactions when contacted with the skin and concerns for its potential toxicity, TPA has not been shown to be an effective tool for treating, managing, or preventing HIV or AIDS.

[0005] Current therapeutics for cytopathic diseases such as various forms of neoplastic disease and viral diseases such as HIV and AIDS suffer from a number of drawbacks such as insufficient potency and intolerable side effects. For many patients, toxic side effects of diminish their quality of life to such an extent they simply stop taking their medications. For others, therapeutic schedules are so complicated and inconvenient that compliance is limited. Other patients experience excellent results initially, but suffer relapses despite full compliance with therapeutic regimens.

[0006] Treatment failure in most HIV cases is attributed to the emergence of resistant strains of HIV. Incomplete viral suppression caused by insufficient drug potency, poor compliance due to complicated drug regimens, and other factors contribute to this problem. Additionally, during the long period of clinical latency of HIV infection, a subset of quiescent memory CD4 T-cells harbor integrated but transcriptionally silent proviruses. This reservoir protects latent HIV from retroviral therapy and poses a substantial barrier to eradication of HIV in infected patients.

[0007] Cancer treatments generally involve a combination of surgery, chemotherapy, hormonal therapy and/or radiation treatment to eradicate neoplastic cells in a patient. However, all of these approaches pose significant drawbacks and added risks such as increased susceptibility to infection. Surgery, for example, may be contraindicated due to the health of a patient. Additionally, it may be difficult to obtain clear margins around a tumor, resulting in some neoplastic tissue being left behind and an increased chance of recurrence of the disease. Almost all current chemotherapeutic agents are toxic, and chemotherapy causes significant side effects including severe nausea, bone marrow depression, and immunosuppression. They also cannot be specifically targeted to cancer cells and therefore may kill healthy cells as well as cancerous ones. Additionally, there are frequently relapsed/refractory neoplasms which are resistant to current therapeutics.

[0008] There is clearly a need for new and more effective treatments for individuals suffering from cytopathic disorders, including those caused by neoplastic disease as well as viral infections such as HIV and AIDS.

Summary of the Exemplary Embodiments of the Invention

[0009] The present invention relates to compositions containing and methods of using phorbol esters in the treatment of cytopathic diseases.

[0010] In one embodiment, phorbol esters and derivatives of phorbol esters are used to treat cytopathic diseases such as HIV and associated conditions such as AIDS. The compositions and methods of the present invention may accomplish the treatment of HIV and associated conditions such as AIDS by any means possible. In some embodiments, the compositions and methods may modify HIV receptor activity in mammalian subjects.

[0011] In another embodiment, compositions and methods may decrease the number of latent HIV reservoirs in an HIV-infected subject. In a further embodiment, it may enhance HIV activation in latent pro-viral cells. In additional embodiments, it may inhibit HIV-cytopathic effects.

[0012] In another embodiment, compositions containing phorbol esters and phorbol ester derivatives may be used for treating and managing symptoms of HIV and AIDS in mammalian subjects. Targeted symptoms for treatment and management employing the compositions and methods of the invention include, but are not limited to, oral lesions, fatigue, skin thrush, fever, lack of appetite, diarrhea, apthous ulcers, malabsorbtion, thrombocytopenia, weight loss, anemia, lymph node enlargement, susceptibility to and severity of secondary conditions such as *mycobacterium avium* complex, salmonellosis, syphilis, neuroshyphilis, turberculosis (TB), bacillary angiomatosis, aspergillosis, candidiasis, coccidioidomycosis, listeriosis, pelvic inflammatory disease, Burkitt's lymphoma, cryptococcal meningitis, histoplasmosis, Kaposi's sarcoma, lymphoma, systemic non-Hodgkin's lymphoma (NHL), primary CNS lymphoma, cryptosporidiosis, isosporiasis, microsporidiosis, *pneumocystis carinii* pneumonia (PCP), toxoplasmosis, cytomegalovirus (CMV), hepatitis, herpes simplex, herpes zoster, human papiloma virus (HPV, genital warts, cervical cancer), molluscum con-

tagiosum, oral hairy leukoplakia (OHL), and progressive multifocal leukoencephalopathy (PML).

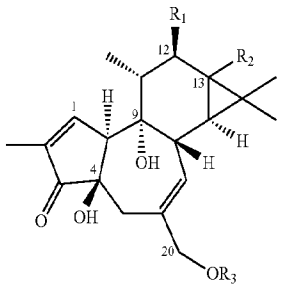
[0013] In a further embodiment, compounds containing phorbol esters and derivatives of phorbol esters may be used to treat cytopathic conditions such as neoplastic diseases. Such neoplasms may be malignant or benign. In some embodiments, neoplasms may be solid or non-solid cancers. In other embodiments, the neoplasms may be relapses. In another embodiment, the neoplasms may be refractory. Exemplary neoplasms include, but are not limited to, hematologic malignancies/bone marrow disorders, including, but not limited to, leukemia, including acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic myeloid leukemia blast crisis, myelodysplasia, and myeloproliferative syndrome; lymphoma, including Hodgkins and non-Hodgkins lymphoma; subcutaneous adenocarcinoma; ovarian teratocarcinoma; and prostate cancer. Other neoplastic conditions amenable to treatment using the methods and compositions include other cancer disorders and conditions, including solid tumors of various types, where successful treatment and/or remission will be determined according to conventional methods, such as determining size reduction of solid tumors, and/or histopathological studies to assess growth, stage, metastatic state or potential, presence or expression levels of histological cancer markers, etc.

[0014] Compositions and methods herein may additionally be used treat symptoms of neoplastic disease including, but not limited to, anemia; chronic fatigue; excessive or easy bleeding, such as bleeding of the nose, gums, and under the skin; easy bruising, particularly bruising with no apparent cause; shortness of breath; petechiae; recurrent fever; swollen gums; slow healing of cuts; bone and joint discomfort; recurrent infections; weight loss; itching; night sweats; lymph node swelling; fever; abdominal pain and discomfort; disturbances in vision; coughing; loss of appetite; pain in the chest; difficulty swallowing; swelling of the face, neck and upper extremities; a need to urinate frequently, especially at night; difficulty starting urination or holding back urine; weak or interrupted flow of urine; painful or burning urination; difficulty in having an erection; painful ejaculation; blood in urine or semen; frequent pain or stiffness in the lower back, hips, or upper thighs; and weakness.

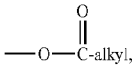
[0015] In yet another embodiment, the phorbol esters and derivatives of phorbol esters may be used to modulate cell signaling pathways. Such modulation may have a variety of results, for example, in some embodiments, the use of compositions containing phorbol esters and derivatives of phorbol esters may increase white blood cell counts in mammalian subjects. In another embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the release of Th1 cytokines in mammalian subjects. In a further embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the release of interleukin 2 (IL-2) in mammalian subjects. In an additional embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the release of interferon in mammalian subjects. In yet another embodiment, compositions containing phorbol esters and/or phorbol ester derivatives may alter the rate of ERK phosphorylation.

[0016] The invention achieves the foregoing and satisfies additional objects and advantages by providing novel and surprisingly effective methods and compositions for modulating cell signaling pathways and/or treating cytopathic diseases and symptoms of cytopathic diseases or conditions using compositions containing a phorbol ester or derivative composition of the Formula I, below:

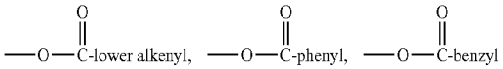
Formula I



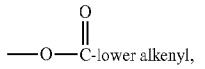
wherein R_1 and R_2 may be hydrogen;



wherein the alkyl group contains 1 to 15 carbon atoms;

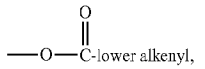


and substituted derivatives thereof and R_3 may be hydrogen or

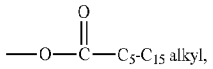


and substituted derivatives thereof.

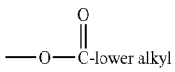
[0017] In another embodiment, at least one of R_1 and R_2 are other than hydrogen and R_3 *is hydrogen or*



and substituted derivatives thereof. In yet another embodiment, either R_1 or R_2 is



the remaining R_1 or R_2 is



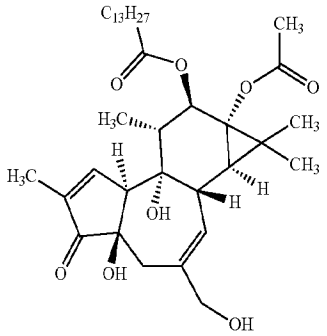
and R_3 is hydrogen.

[0018] The alkyl, alkenyl, phenyl and benzyl groups of the formulas herein may be unsubstituted or substituted with

halogens, preferably, chlorine, fluorine or bromine; nitro; amino; and/or similar type radicals.

[0019] In a further embodiment, the invention achieves these objects and satisfies additional objects and advantages by providing novel and surprisingly effective methods and compositions for modulating cell signaling pathways and/or treating cytopathic diseases or conditions associated with cytopathic diseases using an exemplary phorbol ester composition such as 12-O-tetradecanoylphorbol-13-acetate (TPA) of Formula II, below:

Formula II



[0020] Useful phorbol esters and related compounds and derivatives within the formulations and methods of the invention include, but are not limited to, other pharmaceutically acceptable active salts of said compounds, as well as active isomers, enantiomers, polymorphs, glycosylated derivatives, solvates, hydrates, and/or prodrugs of said compounds. Exemplary forms of phorbol esters for use within the compositions and methods of the invention include, but are not limited to, phorbol 13-butyrate; phorbol 12-decanoate; phorbol 13-decanoate; phorbol 12,13-diacetate; phorbol 13,20-diacetate; phorbol 12,13-dibenzoate; phorbol 12,13-dibutyrate; phorbol 12,13-didecanoate; phorbol 12,13-dihexanoate; phorbol 12,13-dipropionate; phorbol 12-myristate; phorbol 13-myristate; phorbol 12-myristate-13-acetate (also known as TPA or PMA); phorbol 12,13,20-triacetate; 12-deoxyphorbol 13-angelate; 12-deoxyphorbol 13-angelate 20-acetate; 12-deoxyphorbol 13-isobutyrate; 12-deoxyphorbol 13-isobutyrate-20-acetate; 12-deoxyphorbol 13-phenylacetate; 12-deoxyphorbol 13-phenylacetate 20-acetate; 12-deoxyphorbol 13-tetradecanoate; phorbol 12-tigliate 13-decanoate; 12-deoxyphorbol 13-acetate; phorbol 12-acetate; and phorbol 13-acetate.

[0021] In exemplary embodiments, the compositions and methods of the invention employ a phorbol ester compound of Formula I to treat and/or prevent symptoms of cytopathic diseases including, but not limited to, symptoms of HIV and AIDS or other diseases and conditions associated with HIV and AIDS such as opportunistic infections, as well as symptoms of neoplastic diseases or other diseases and conditions associated with neoplastic diseases.

[0022] Mammalian subjects amenable to treatment with phorbol esters of Formula I, particularly TPA, according to the methods of the invention include, but are not limited to, subjects with HIV and AIDS, as well as subjects with symptoms, or secondary or opportunistic diseases associated with HIV and AIDS, such as oral lesions, fatigue, skin thrush, fever, lack of appetite, diarrhea, apthous ulcers, malabsorption, thrombocytopenia, weight loss, anemia, lymph node enlargement, *mycobacterium avium* complex, salmonellosis, syphilis, neuroshyphilis, turberculosis (TB), bacillary angiomatosis, aspergillosis, candidiasis, coccidioidomycosis, listeriosis, pelvic inflammatory disease, Burkitt's lymphoma, cryptococcal meningitis, histoplasmosis, Kaposi's sarcoma, lymphoma, systemic non-Hodgkin's lymphoma (NHL), primary CNS lymphoma, cryptosporidiosis, isosporiasis, microsporidiosis, *pneumocystis carinii* pneumonia (PCP), toxoplasmosis, cytomegalovirus (CMV), hepatitis, herpes simplex, herpes zoster, human papiloma virus (HPV, genital warts, cervical cancer), molluscum contagiosum, oral hairy leukoplakia (OHL), and progressive multifocal leukoencephalopathy (PML).

[0023] Additional mammalian subjects amenable to treatment with phorbol esters of Formula I, particularly TPA, according to the methods of the present invention include, but are not limited to, subjects suffering from neoplastic diseases including malignant neoplastic diseases such as solid and non-solid cancers. Non-solid cancers may include, hematologic malignancies/bone marrow disorders, including, but not limited to, leukemia, including acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic myeloid leukemia blast crisis, myelodysplasia, myeloproliferative syndrome. Solid cancers may include, but are not limited to, lymphoma, including Hodgkins and non-Hodgkins lymphoma, subcutaneous adenocarcinoma, ovarian teratocarcinoma, and prostate cancer. Subjects amenable to treatment with phorbol esters of Formula I, particularly TPA additionally include those suffering from symptoms of such neoplastic diseases such as, but not limited to, anemia; chronic fatigue; excessive or easy bleeding, such as bleeding of the nose, gums, and under the skin; easy bruising, particularly bruising with no apparent cause; shortness of breath; petechiae; recurrent fever; swollen gums; slow healing of cuts; bone and joint discomfort; recurrent infections; weight loss; itching; night sweats; lymph node swelling; fever; abdominal pain and discomfort; disturbances in vision; coughing; loss of appetite; pain in the chest; difficulty swallowing; swelling of the face, neck and upper extremities; a need to urinate frequently, especially at night; difficulty starting urination or holding back urine; weak or interrupted flow of urine; painful or burning urination; difficulty in having an erection; painful ejaculation; blood in urine or semen; frequent pain or stiffness in the lower back, hips, or upper thighs; and weakness. In some embodiments, such cancers may be relapses or refractory.

[0024] These and other subjects are effectively treated, prophylactically and/or therapeutically, by administering to the subject an effective amount of a phorbol ester of Formula I sufficient to prevent or reduce viral load, decrease latent reservoirs of HIV, increase immune responsiveness, increase the release of Th1 cytokines, prevent or reduce symptoms and conditions associated with HIV and AIDS, decrease and/or eliminate neoplastic cells, increase white blood cell counts, induce remission, maintain remission, prevent or reduce symptoms and conditions associated with malignancies and/or increase ERK phosphorylation. Therapeutically useful methods and formulations of the invention will effectively use a phorbol ester of Formula I in a variety of forms, as noted above, including any active, pharmaceutically acceptable salts of said compounds, as well as active isomers, enantiomers, polymorphs, solvates, hydrates, prodrugs, and/or

combinations thereof. TPA of formula II is employed as an illustrative embodiment of the invention within the examples herein below.

[0025] Within additional aspects of the invention, combinatorial formulations and methods are provided which employ an effective amount of a phorbol ester of Formula I in combination with one or more secondary or adjunctive active agent(s) that is/are combinatorially formulated or coordinately administered with the phorbol ester compound of Formula I to yield an effective response in the subject. Exemplary combinatorial formulations and coordinate treatment methods in the treatment of viral cytopathic diseases such as HIV and AIDS employ the phorbol ester compound of Formula I in combination with one or more additional, retroviral, HIV or AIDS treating or other indicated secondary or adjunctive therapeutic agents. Such combinatorial formulations and coordinate treatment methods may, for example, follow or be derived from various highly active antiretroviral therapy protocols (HAART protocols) and include regimens such as, but not limited to, two nucleoside analogue reverse transcriptase inhibitors plus one or more protease inhibitor or non-nucleoside analogue reverse transcriptase inhibitor among other combinations. Other combinatorial formulations and coordinate treatment methods may, for example, include treatments for opportunistic infections as well as the compounds for the HAART protocols. The secondary or adjunctive therapeutic agents used in combination with, e.g., TPA, in these embodiments may possess direct or indirect antiviral effects, alone or in combination with, e.g. TPA, may exhibit other useful adjunctive therapeutic activity in combination with, e.g. TPA (such as HIV preventing, HIV treating, HIV reservoir activating, Th1 cytokine increasing activity); or may exhibit adjunctive therapeutic activity useful for treating opportunistic infections associated with HIV alone or in combination with, e.g. TPA.

[0026] Useful adjunctive therapeutic agents in these combinatorial formulations and coordinate treatment methods include, for example, protease inhibitors, including, but not limited to, saquinavir, indinavir, ritonavir, nelfinavir, atazanavir, darunavir, fosamprenavir, tipranavir and amprenavir; nucleoside reverse transcriptase inhibitors including but not limited to, zidovudine, didanosine, stavudine, lamivudine, zalcitabine, emtricitabine, tenofovir disoproxil fumarate, AVX754 and abacavir; non-nucleoside reverse transcriptase inhibitors including, but not limited to, nevaripine, delavirdine, calanolide A, TMC 125 and efavirenz; combination drugs including, but not limited to, efavirenz/emtricitabine/tenofovir disoproxil fumarate, lamivudine/zidovudine, abacavir/lamivudine, abacavir/lamivudine/zidovudine, emtricitabine/tenofovir disoproxil fumarate, sulfamethoxazole/trimethoprim, and lopinavir/ritonavir; entry and fusion inhibitors, including, but not limited to, enfuvirtide, AMD070, BMS-488043, fozivudine tidoxil, GSK-873,140, PRO140, PRO542, Peptide T, SCH-D, TNX-355, and UK-427,857; treatments for opportunistic infections and other conditions associated with AIDS and HIV including, but not limited to, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, paclitaxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, and valganciclovir; integrase inhibitors including, but not limited to, GS 9137, MK-0518; microbicides, including, but not limited to, BMS-378806, C31G, carbopol 974P, carrageenan, cellulose sulfate, cyanovirin-N, dextran sulfate, hydroxyethyl cellulose, PRO2000, SPL7013, tenofovir, UC-781 and IL-2.

[0027] Exemplary combinatorial formulations and coordinate treatment methods in the treatment of neoplastic disease employ the phorbol ester compound of Formula I in combination with one or more additional, neoplastic disease treating or other indicated, secondary or adjunctive therapeutic agents. The secondary or adjunctive therapeutic agents used in combination with, e.g., TPA, in these embodiments may possess direct or indirect chemotherapeutic effects, alone or in combination with, e.g. TPA, may exhibit other useful adjunctive therapeutic activity in combination with, e.g. TPA (such as cytotoxic, anti-inflammatory, NF-κB inhibiting, apoptosis inducing, Th1 cytokine increasing activity); or may exhibit adjunctive therapeutic activity useful for treating neoplasms or associated symptoms alone or in combination with, e.g. TPA.

[0028] Useful adjunctive or secondary therapeutic agents in these combinatorial formulations and coordinate treatment methods include doxorubicin, vitamin D3, cytarabine, cytosine arabinoside, daunorubicin, cyclophosphamide, gemtuzumab ozogamicin, idarubicin, mercaptopurine, mitoxantrone, thioguanine, aldesleukin, asparaginase, carboplatin, etoposide phosphate, fludarabine, methotrexate, etoposide, dexamethasone, and choline magnesium trisalicylate. In addition, adjunctive or secondary therapies may be used such as, but not limited to, radiation treatment, hormone therapy and surgery.

[0029] The forgoing and additional objects, features, aspects and advantages of the present invention will become apparent from the following detailed description.

Detailed Description of Exemplary Embodiments of the Invention

[0030] Novel methods and compositions have been identified for use in preventing and/or treating cytopathic diseases and conditions in mammalian subjects. In various embodiments, the methods and compositions are effective to prevent or treat HIV and AIDS and related conditions, diseases caused by HIV and AIDS, and/or diseases acquired because of HIV or AIDS infection. In other embodiments, the methods and compositions are effective to prevent or treat neoplastic diseases and symptoms of such diseases. Such neoplastic diseases may or may not be malignant. In some embodiments, the neoplastic diseases may be solid or non-solid cancers. In other embodiments, the cancers may be refractory or relapses.

[0031] Formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as novel HIV and AIDS treating compounds.

[0032] Formulations and methods provided herein additionally employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, iso-

mers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof in the treatment of neoplastic diseases.

[0033] Viral load decreasing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as novel viral load decreasing agents.

[0034] Apoptosis inducing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as chemotherapeutic agents that induce apoptosis in neoplasms.

[0035] Remission inducing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as anti-neoplasm agents.

[0036] Immune responsiveness increasing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as immune stimulatory compounds.

[0037] Th1 cytokine increasing formulations and methods provided herein employ a phorbol ester or derivative compound of Formula I, above, including all active pharmaceutically acceptable compounds of this description as well as various foreseen and readily provided complexes, salts, solvates, isomers, enantiomers, polymorphs and prodrugs of these compounds and combinations thereof as novel Th1 cytokine increasing agents.

[0038] A broad range of mammalian subjects, including human subjects, are amenable to treatment using the formulations and methods of the invention. These subjects include, but are not limited to, individuals suffering from cytopathic diseases or conditions including neoplastic diseases and viral cytopathic diseases such as HIV and AIDS.

[0039] Subjects amenable to treatment include HIV+ human and other mammalian subjects presenting with oral lesions, fatigue, skin thrush, fever, lack of appetite, diarrhea, apthous ulcers, malabsorption, thrombocytopenia, weight loss, anemia, lymph node enlargement, susceptibility to and severity of secondary conditions such as *mycobacterium avium* complex, salmonellosis, syphilis, neuroshyphilis, turberculosis (TB), bacillary angiomatosis, aspergillosis, candidiasis, coccidioidomycosis, listeriosis, pelvic inflammatory disease, Burkitt's lymphoma, cryptococcal meningitis, histoplasmosis, Kaposi's sarcoma, lymphoma, systemic non-Hodgkin's lymphoma (NHL), primary CNS lymphoma, cryptosporidiosis, isosporiasis, microsporidiosis, *pneumocystis carinii* pneumonia (PCP), toxoplasmosis, cytomegalovirus (CMV), hepatitis, herpes simplex, herpes zoster, human papiloma virus (HPV, genital warts, cervical cancer), molluscum contagiosum, oral hairy leukoplakia (OHL), and progressive multifocal leukoencephalopathy (PML).

[0040] Within the methods and compositions of the invention, one or more phorbol ester compound(s) of Formula I as disclosed herein is/are effectively formulated or administered as an agent effective for treating HIV/AIDS and/or related disorders. In exemplary embodiments, TPA is demonstrated for illustrative purposes to be an effective agent in pharmaceutical formulations and therapeutic methods, alone or in combination with one or more adjunctive therapeutic agent (s). The present disclosure further provides additional, pharmaceutically acceptable phorbol ester compounds in the form of a native or synthetic compound, including complexes, derivatives, salts, solvates, isomers, enantiomers, polymorphs, and prodrugs of the compounds disclosed herein, and combinations thereof, which are effective as therapeutic agents within the methods and compositions of the invention in the treatment of HIV/AIDS and related conditions.

[0041] Acquired immune deficiency syndrome or acquired immunodeficiency syndrome (AIDS or Aids) is a collection of symptoms and infections resulting from damage to the immune system caused by infection with the human immunodeficiency virus (HIV). The damage to the immune system leaves individuals prone to opportunistic infections and tumors. Although treatments for AIDS and HIV exist to slow the virus's progression and the severity of the symptoms, there is no known cure.

[0042] HIV is a retrovirus that primarily infects components of the human immune system such as CD4+ T cells, macrophages and dendritic cells. When CD4+ T cells are destroyed and their total count decreases to below 200 CD4+ T cells/μL of blood or the percentage of CD4+ T-cell as a fraction of the total lymphocytes falls to less than 14%, cellular immunity is lost, leading to AIDS.

[0043] It is currently believed that a change in the T_h1 and T_h2 cytokine balance can contribute to immune dysregulation associated with HIV infection. T_h1 cells produce cytokines that stimulate proliferation of cytotoxic T cells. T_h2 cells produce cytokines that are responsible for activation of the humoral immune responses in healthy people. Progression from HIV infection to AIDS is characterized by a decrease in levels of T_h1 cytokines IL-2, IL-12 and IFN-γ with a concomitant increase in levels of T_h2 cytokines IL-4, IL-5 and IL-10. (Clerci, Immunology Today, v. 14, No. 3, p. 107-110, 1993; Becker, Virus Genes 28:1, 5-18 (2004)). Resistance to HIV infection and/or resistance to progression to AIDS may therefore be dependent on a $T_h1 > T_h2$ dominance.

[0044] A fraction of CD4+ memory T cells contain integrated transcritpionally inactive proviruses for HIV. These latent reservoirs may be activated to produce active infectious virus following activation by specific antigens or cytokines. The half life of these CD4 memory T cells is at least 44 months making it extremely difficult to eliminate HIV and requiring extended continuation of antiretroviral therapy even when HIV levels in the peripheral blood are undetectable.

[0045] Prostratin, 12-deoxyphorbol 13-acetate, a non-tumor promoting phorbol ester, has reportedly shown some effectiveness for inhibiting HIV induced cell killing and viral replication. Prostratin reportedly activated viral expression in latently-infected cell lines, but had little or no effect on chronically-infected cell lines. (Gulakowski, et al., Antiviral Research v. 33, 87-97 (1997); Williams, et al., JBC v. 279, No. 40, P. 42008-42017 (2004)). Prostratin represents a distinct

subclass of protein kinase C activators which has unique biological activities that differ from tumor-promoting phorbol esters such as TPA.

[0046] Mammalian subjects amenable to treatment with phorbol esters of Formula I, particularly TPA, according to the methods of the present invention additionally include, but are not limited to, mammalian subjects with neoplastic diseases including solid and non-solid cancers, including hematologic malignancies/bone marrow disorders, such as leukemia, including acute myeloid leukemia (AML), chronic myeloid leukemia(CML), chronic myeloid leukemia blast crisis, myelodysplasia, myeloproliferative syndrome; lymphoma, including Hodgkins and non-Hodgkins lymphoma; subcutaneous adenocarcinoma; ovarian teratocarcinoma; and prostate cancer. In some embodiments, such cancers may be relapses or refractory.

[0047] Within the methods and compositions of the invention, one or more phorbol ester compound(s) of Formula I as disclosed herein is/are effectively formulated or administered as an agent effective for treating neoplastic diseases. In exemplary embodiments, TPA is demonstrated for illustrative purposes to be an effective agent in pharmaceutical formulations and therapeutic methods, alone or in combination with one or more adjunctive therapeutic agent(s). The present disclosure further provides additional, pharmaceutically acceptable phorbol ester compounds in the form of a native or synthetic compound, including complexes, derivatives, salts, solvates, isomers, enantiomers, polymorphs, and prodrugs of the compounds disclosed herein, and combinations thereof, which are effective as therapeutic agents within the methods and compositions of the invention in the treatment of neoplastic diseases and symptoms of such diseases.

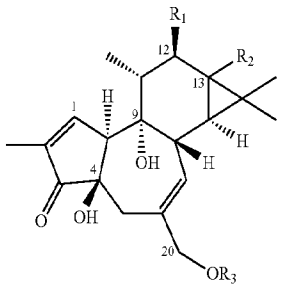
[0048] Neoplastic disease is any growth or tumor caused by abnormal and uncontrolled cell division; it may spread to other parts of the body through the lymphatic system or the blood stream. Such growths may be malignant or benign, solid or non-solid.

[0049] In some embodiments, the neoplastic diseases may be a hematological neoplasm/bone marrow disorder such as acute myeloid leukemia (AML). AML (also called acute myelogenous leukemia, acute myeloblastic leukemia, acute granulocytic leukemia, and acute nonlymphocytic leukemia) is the most common type of acute leukemia in adults. In AML, stem cells produced by the bone marrow usually develop into a type of immature white blood cell called myeloblasts (or myeloid blasts). In individuals suffering from AML, these myeloblasts do not mature into healthy white blood cells. Additionally, stem cells in individuals with AML may develop into abnormal red blood cells or platelets. The lack of normal blood cells increases incidences of infection, anemia, and easy bleeding. Additionally, the leukemia cells can spread outside the blood to other parts of the body, including the central nervous system (brain and spinal cord), skin, and gums.

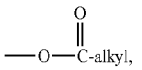
[0050] The average age of a patient with AML is over 64 years of age. Patients over the age of 60 treated for AML with standard chemotherapeutics have a remission rate of less than 20%. Additionally, patients who develop AML after an antecedent hematologic disorder or prior leukemogenic chemotherapy/radition therapy have similarly poor outcomes.

[0051] Phorbol is a natural, plant-derived polycyclic alcohol of the tigliane family of diterpenes. It was first isolated in 1934 as the hydrolysis product of *croton* oil derived from the seeds of *Croton tiglium*. It is well soluble in most polar organic solvents and in water. Esters of phorbol have the general structure of Formula I, below:

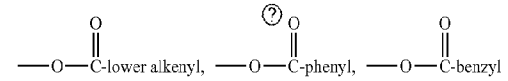
Formula I



wherein R_1 and R_2 are selected from the group consisting of hydrogen;



wherein the alkyl group contains 1 to 15 carbon atoms,



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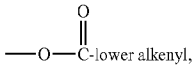
and substituted derivatives thereof and R_3 may be hydrogen,



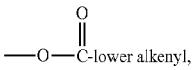
or substituted derivatives thereof.

[0052] The term "lower alkyl" or "lower alkenyl" as used herein means moieties containing 1-7 carbon atoms. In the compounds of the Formula I, the alkyl or alkenyl groups may be straight or branched chain. In some embodiments, either or both R_1 or R_2, are a long chain carbon moiety (i.e., Formula I is decanoate or myristate).

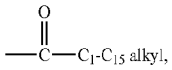
[0053] The alkyl, alkenyl, phenyl and benzyl groups of the formulas herein may be unsubstituted or substituted with halogens, preferably, chlorine, fluorine or bromine; nitro; amino and similar type radicals.

[0054] Organic and synthetic forms of phorbol esters, including any preparations or extracts from herbal sources such as *croton* tiglium, are contemplated as useful compositions comprising phorbol esters (or phorbol ester analogs, related compounds and/or derivatives) for use within the embodiments herein. Useful phorbol esters and/or related compounds for use within the embodiments herein will typically have a structure as illustrated in Formula I, although functionally equivalent analogs, complexes, conjugates, and derivatives of such compounds will also be appreciated by those skilled in the art as within the scope of the invention.

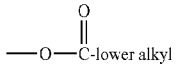
[0055] In more detailed embodiments, illustrative structural modifications according to Formula I above will be selected to provide useful candidate compounds for treating and/or preventing HIV and AIDS and/or neoplastic diseases, wherein: at least one of R_1 and R_2 are other than hydrogen and R_3 is selected from the group consisting of hydrogen



and substituted derivatives thereof. In another embodiment, either R_1 or R_2 is



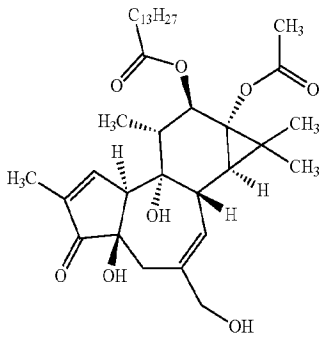
the remaining R_1 or R_2 is



and R_3 is hydrogen.

[0056] An exemplary embodiment of a phorbol ester compound of Formula I useful in the treatment of cytopathic diseases such as HIV and AIDS and/or neoplastic diseases, particularly AML, is found in phorbol 12-myristate-13-acetate (also known as PMA or 12-O-tetradecanoyl-phorbol-13-acetate (TPA)) shown in Formula II, below.

Formula II



[0057] Additional useful phorbol esters and related compounds and derivatives within the formulations and methods of the invention include, but are not limited to, other pharmaceutically acceptable active salts of said compounds, as well as active isomers, enantiomers, polymorphs, glycosylated derivatives, solvates, hydrates, and/or prodrugs of said compounds. Further exemplary forms of phorbol esters for use within the compositions and methods of the invention include, but are not limited to, phorbol 13-butyrate; phorbol 12-decanoate; phorbol 13-decanoate; phorbol 12,13-diacetate; phorbol 13,20-diacetate; phorbol 12,13-dibenzoate; phorbol 12,13-dibutyrate; phorbol 12,13-didecanoate; phorbol 12,13-dihexanoate; phorbol 12,13-dipropionate; phorbol 12-myristate; phorbol 13-myristate; phorbol 12,13,20-triacetate; 12-deoxyphorbol 13-angelate; 12-deoxyphorbol 13-angelate 20-acetate; 12-deoxyphorbol 13-isobutyrate; 12-deoxyphorbol 13-isobutyrate-20-acetate; 12-deoxyphorbol 13-phenylacetate; 12-deoxyphorbol 13-phenylacetate 20-acetate; 12-deoxyphorbol 13-tetradecanoate; phorbol 12-tigliate 13-decanoate; 12-deoxyphorbol 13-acetate; phorbol 12-acetate; and phorbol 13-acetate.

[0058] Cytopathic disease treating compositions herein comprise HIV- and AIDS-treating compositions comprising an anti-AIDS effective amount of a phorbol ester compound of Formula I, which is effective for prophylaxis and/or treatment of HIV, AIDS, and/or HIV-related symptoms, including opportunistic infections, in a mammalian subject. An "anti-HIV", "anti-AIDS", or "AIDS treating" effective amount of the active compound is therapeutically effective, in a single or multiple unit dosage form, over a specified period of therapeutic intervention, to measurably alleviate one or more symptoms of AIDS in a subject, and/or to alleviate one or more symptom(s) or condition(s) associated with HIV infection in the subject. Within exemplary embodiments, the compositions of the invention are effective in treatment methods to alleviate symptoms of AIDS or other HIV-related conditions in human and other mammalian subjects vulnerable to HIV infection.

[0059] Cytopathic disease treating compositions herein additionally may comprise chemotherapeutic compositions comprising an anti-neoplastic effective amount of a phorbol ester or derivative compound of Formula I, which is effective for maintenance and treatment of malignancies or symptoms caused by cancer in a mammalian subject. A "chemotherapeutic", "anti-tumor," "cancer treating", "apoptosis inducing", "remission inducing", "remission maintaining" effective amount of the active compound is therapeutically effective, in a single or multiple unit dosage form, over a specified period of therapeutic intervention, to measurably alleviate one or more symptoms of malignancy in a subject, and/or to alleviate one or more symptom(s) or condition(s) associated with malignancy in the subject. Within exemplary embodiments, the compositions of the invention are effective in treatment methods to alleviate symptoms of neoplastic disease related conditions in human and other mammalian subjects vulnerable to malignancies.

[0060] Cytopathic disease treating, including chemotherapeutic and HIV treating, compositions of the invention typically comprise an effective amount or unit dosage of a phorbol ester compound of Formula I, which may be formulated with one or more pharmaceutically acceptable carriers, excipients, vehicles, emulsifiers, stabilizers, preservatives, buffers, and/or other additives that may enhance stability, delivery, absorption, half-life, efficacy, pharmacokinetics, and/or pharmacodynamics, reduce adverse side effects, or provide other advantages for pharmaceutical use. Effective amounts of a phorbol ester compound or related or derivative compound of Formula I (e.g., a unit dose comprising an effective concentration/amount of TPA, or of a selected pharmaceutically acceptable salt, isomer, enantiomer, solvate, polymorph and/or prodrug of TPA) will be readily determined by those of ordinary skill in the art, depending on clinical and patient-specific factors. Suitable effective unit dosage amounts of the active compounds for administration to mammalian subjects, including humans, may range from 10 to 1500 μg, 20 to 1000 μg, 25 to 750 μg, 50 to 500 μg, or 150 to 500 μg. In certain embodiments, the cytopathic disease treating effective dosage of a phorbol ester compound or

related or derivative compound of Formula I may be selected within narrower ranges of, for example, 10 to 25 μg, 30-50 μg, 75 to 100 μg, 100 to 250 μg, or 250 to 500 μg. These and other effective unit dosage amounts may be administered in a single dose, or in the form of multiple daily, weekly or monthly doses, for example in a dosing regimen comprising from 1 to 5, or 2 to 3, doses administered per day, per week, or per month. In one exemplary embodiment, dosages of 10 to 30 μg, 30 to 50 μg, 50 to 100 μg, 100 to 250 μg, or 250 to 500 μg, are administered one, two, three, four, or five times per day. In more detailed embodiments, dosages of 50-100 μg, 100-250 μg, 250-400 μg, or 400-600 μg are administered once or twice daily. In a further embodiment, dosages of 50-100 μg, 100-2500 μg, 250-400 μg, or 400-600 μg are administered every other day. In alternate embodiments, dosages are calculated based on body weight, and may be administered, for example, in amounts from about 0.5 μg/sq·m to about 100 μg/sq·m per day, 1 μg/sq·m to about 75 μg/sq·m per day, 1 μg/sq·m to about 50 μg/sq·m per day, 2 μg/sq·m to about 50 μg/sq·m per day, 2 μg/sq·m to about 30 μg/sq·m per day or 3 μg/sq·m to about 30 μg/sq·m per day.

[0061] The amount, timing and mode of delivery of compositions of the invention comprising a cytopathic disease treating effective amount of a phorbol ester compound of Formula I (AIDS treating, HIV preventing, HIV treating, HIV reservoir activating, Th1 cytokine increasing, ERK phosphorylation inducing, chemotherapeutic, anti-tumor, cancer treating, remission inducing, remission maintaining, apoptosis inducing effective amount) will be routinely adjusted on an individual basis, depending on such factors as weight, age, gender, and condition of the individual, the acuteness of the cytopathic disease and/or related symptoms, whether the administration is prophylactic or therapeutic, and on the basis of other factors known to effect drug delivery, absorption, pharmacokinetics, including half-life, and efficacy.

[0062] An effective dose or multi-dose treatment regimen for the instant cytopathic disease treating (alternatively, "AIDS treating", "HIV treating", "HIV preventing", "HIV reservoir activating", or "Th1 cytokine increasing", "ERK phosphorylation inducing", "chemotherapeutic", "anti-tumor", "cancer treating", "apoptosis inducing", "remission inducing", "remission maintaining") formulations of the invention will ordinarily be selected to approximate a minimal dosing regimen that is necessary and sufficient to substantially prevent or alleviate the symptoms of the cytopathic disease including AIDS or neoplastic diseases such as cancer and related opportunistic diseases in the subject, and/or to substantially prevent or alleviate one or more symptoms associated with AIDS or neoplastic diseases such as cancer in the subject. A dosage and administration protocol will often include repeated dosing therapy over a course of several days or even one or more weeks or years. An effective treatment regime may also involve prophylactic dosage administered on a day or multi-dose per day basis lasting over the course of days, weeks, months or even years.

[0063] Various assays and model systems can be readily employed to determine the therapeutic effectiveness of the treatment of cytopathic diseases. For example in the treatment of HIV or AIDS effectiveness may be demonstrated by a decrease in viral load, an increase in CD4 counts, an increase in CD3 counts, an increase in IL-2 and IFN production, a decrease in IL-4 and IL-10 production, and a decrease or elimination of the symptoms of AIDS among other methods of determining effectiveness known to those of skill in the art.

[0064] Effectiveness of the compositions and methods of the invention may be demonstrated, for example, through blood tests for HIV antibodies, viral load, CD4 levels, CD8 counts, and CD3 counts. Normal levels of CD4 are usually between 600 and 1200 per microliter, or 32-68% of lymphocytes. Individuals with a CD4 count of less than 350 have a weakened immune system. Those with a CD4 count of less than 200 are considered to have AIDS. CD8 levels in a healthy individual are generally between 150-1000 per microliter. CD3 levels in a healthy individual are generally between about 885-2270 per microliter. Levels of CD3, CD4 and CD8 cells may be measured, for example, using flow cytometry. Effective amounts of the compositions of the invention will increase levels of CD3, CD4 and CD8 positive cells by at least 10%, 20%, 30%, 50% or greater reduction, up to a 75-90%, or 95% or greater. Effective amounts will also move the CD3, CD4 and CD8 profile of an individual towards the optimal category for each type of glycoprotein.

[0065] Individuals may also be evaluated using a $beta_2$-microglobulin (beta $_2$-M) test. $Beta_2$-microglobulin is a protein released into the blood when a cell dies. A rising beta $_2$-M blood level can be used to measure the progression of AIDS. Effective amounts of a composition of the present invention will lead to a decrease or cessation of increase in the amount of beta $_2$-M.

[0066] Effectiveness may further be demonstrated using a complete blood count (CBC). The measurements taken in a CBC include a white blood cell count (WBC), a red blood cell count (RBC), the red cell distribution width, the hematocrit, and the amount of hemoglobin. Specific AIDS-related signs in a CBC include a low hematocrit, a sharp decrease in the number of blood platelets, and a low level of neutrophils. An effective amount of a composition of the present invention will increase the levels measured in a complete blood count by 10%, 20%, 30%, 50% or greater increase, up to a 75-90%, or 95% or greater. Effective amounts will also move the blood protein of an individual towards the optimal category for each type of protein.

[0067] Effectiveness of the compositions and methods of the invention may also be demonstrated by a decrease in the symptoms of HIV or AIDS including, but not limited to, oral lesions, fatigue, skin thrush, fever, lack of appetite, diarrhea, apthous ulcers, malabsorption, thrombocytopenia, weight loss, anemia, and lymph node enlargement.

[0068] Effectiveness of the compositions and methods of the invention may also be demonstrated by a decrease in the susceptibility to and severity of secondary or opportunistic conditions such as *mycobacterium avium* complex, salmonellosis, syphilis, neuroshyphilis, turberculosis (TB), bacillary angiomatosis, aspergillosis, candidiasis, coccidioidomycosis, listeriosis, pelvic inflammatory disease, Burkitt's lymphoma, cryptococcal meningitis, histoplasmosis, Kaposi's sarcoma, lymphoma, systemic non-Hodgkin's lymphoma (NHL), primary CNS lymphoma, cryptosporidiosis, isosporiasis, microsporidiosis, *pneumocystis carinii* pneumonia (PCP), toxoplasmosis, cytomegalovirus (CMV), hepatitis, herpes simplex, herpes zoster, human papiloma virus (HPV, genital warts, cervical cancer), molluscum contagiosum, oral hairy leukoplakia (OHL), and progressive multifocal leukoencephalopathy (PML).

[0069] Effectiveness may further be demonstrated by reduction of detectable HIV in the HIV-infected subject; maintaining a normal T cell count; or maintaining normal p24 antigen levels.

[0070] Effectiveness in the treatment of neoplastic diseases may also be determined by a number of methods such as, but not limited to, ECOG Performance Scale, the Karnofsky Performance Scale, microscopic examination of blood cells, bone marrow aspiration and biopsy, cytogenetic analysis, biopsy, immunophenotyping, blood chemistry studies, a complete blood count, lymph node biopsy, peripheral blood smear, visual analysis of a tumor or lesion, or any other method of evaluating and/or diagnosing malignancies and tumor progression known to those of skill in the art.

[0071] For example, effectiveness of the compositions and methods herein in the treatment of hematologic malignancies/bone marrow disorders may be evaluated using, an absolute neutrophil count (ANC). A normal ANC is between 1,500 to 8,000/mm3. Individuals suffering from hematologic malignancies/bone marrow disorders frequently have an ANC below 1500/mm^3, and may even reach levels below 500/mm^3 Effective amounts of the compositions and methods herein will increase an individual's ANC by 10%, 20%, 30%, 50% or greater increase, up to a 75-90%, or 95% or greater. Effective amounts may increase ANC levels above 1500/mm^3.

[0072] Effectiveness of the compositions and methods herein in the treatment of hematologic malignancies/bone marrow disorders may further be evaluated using, for example, a platelet count. A platelet count is normally between 150,000 to 450,000 platelets per microliter (×10−6/Liter). Individuals suffering from hematologic malignancies/bone marrow disorder may have platelet counts below 100,000 per microliter. Effective amounts of the compositions and methods herein will increase an individual's platelet count by 10%, 20%, 30%, 50% or greater increase, up to a 75-90%, or 95% or greater. Effective amounts may increase platelet levels above 100,000 per microliter.

[0073] Effectiveness of the compositions and methods herein in the treatment of hematologic malignancies/bone marrow disorders may additionally be evaluated, for example, by measuring the number of myeloblasts. Myeloblasts normally represent less than 5% of the cells in the bone marrow but should not be present in circulating blood. Effective amounts of the compositions and methods herein will decrease the number of myeloblasts by 10%, 20%, 30%, 50% or more, up to a 75-90%, 96% or greater decrease. Effective amounts may decrease myeloblasts to below 5%.

[0074] Effectiveness of the compositions and methods herein in the treatment of hematologic malignancies/bone marrow disorders may further be evaluated by examining myeloblasts for the presence of Auer rods. Effective amounts of the compositions of the present invention will decrease the number of Auer rods visible by 10%, 20%, 30%, 50% or more, up to a 75-90%, 96% or greater decrease up to the complete elimination of Auer rods.

[0075] Effectiveness of the compositions and methods of the invention may also be demonstrated by a decrease in the symptoms of subjects suffering from neoplastic disease including, but not limited to, anemia; chronic fatigue; excessive or easy bleeding, such as bleeding of the nose, gums, and under the skin; easy bruising, particularly bruising with no apparent cause; shortness of breath; petechiae; recurrent fever; swollen gums; slow healing of cuts; bone and joint discomfort; recurrent infections; weight loss; itching; night sweats; lymph node swelling; fever; abdominal pain and discomfort; disturbances in vision; coughing; loss of appetite; pain in the chest; difficulty swallowing; swelling of the face, neck and upper extremities; a need to urinate frequently, especially at night; difficulty starting urination or holding back urine; weak or interrupted flow of urine; painful or burning urination; difficulty in having an erection; painful ejaculation; blood in urine or semen; frequent pain or stiffness in the lower back, hips, or upper thighs; and weakness.

[0076] For each of the indicated conditions described herein, test subjects will exhibit a 10%, 20%, 30%, 50% or greater reduction, up to a 75-90%, or 96% or greater, reduction, in one or more symptom(s) caused by, or associated with, the cytopathic disease, or related diseases or conditions in the subject, compared to placebo-treated or other suitable control subjects.

[0077] Within additional aspects of the invention, combinatorial cytopathic disease treating (AIDS treating, HIV preventing, HIV treating, HIV reservoir activating, Th1 cytokine increasing, ERK phosphorylation inducing, apoptosis inducing, chemotherapeutic, anti-tumor, cancer treating, remission inducing, remission maintaining) formulations and coordinate administration methods are provided which employ an effective amount of a phorbol ester compound of Formula I and one or more secondary or adjunctive agent(s) that is/are combinatorially formulated or coordinately administered with the phorbol ester compound of Formula I to yield a combined, multi-active cytopathic disease treating composition or coordinate treatment method.

[0078] Exemplary combinatorial formulations and coordinate treatment methods in this context employ the phorbol ester of Formula I in combination with the one or more secondary anti-AIDS agent(s), or with one or more adjunctive therapeutic agent(s) that is/are useful for treatment or prophylaxis of the targeted (or associated) disease, condition and/or symptom(s) in the selected combinatorial formulation or coordinate treatment regimen. For most combinatorial formulations and coordinate treatment methods of the invention, a phorbol ester compound of Formula I or related or derivative compound is formulated, or coordinately administered, in combination with one or more secondary or adjunctive therapeutic agent(s), to yield a combined formulation or coordinate treatment method that is combinatorially effective or coordinately useful to treat HIV/AIDS and/or one or more symptom(s) of a opportunistic or secondary disease or condition in the subject. Exemplary combinatorial formulations and coordinate treatment methods in this context employ a phorbol ester compound of Formula I in combination with one or more secondary or adjunctive therapeutic agents selected from, e.g., protease inhibitors, including, but not limited to, saquinavir, indinavir, ritonavir, nelfinavir, atazanavir, darunavir, fosamprenavir, tipranavir and amprenavir; nucleoside reverse transcriptase inhibitors including but not limited to, zidovudine, didanosine, stavudine, lamivudine, zalcitabine, emtricitabine, tenofovir disoproxil fumarate, AVX754 and abacavir; non-nucleoside reverse transcriptase inhibitors including, but not limited to, nevaripine, delavirdine, calanolide A, TMC125 and efavirenz; combination drugs including, but not limited to, efavirenz/emtricitabine/tenofovir disoproxil fumarate, lamivudine/zidovudine, abacavir/lamivudine, abacavir/lamivudine/zidovudine, emtricitabine/tenofovir disoproxil fumarate, sulfamethoxazole/trimethoprim, and lopinavir/ritonavir; entry and fusion

inhibitors, including, but not limited to, enfuvirtide, AMD070, BMS-488043, fozivudine tidoxil, GSK-873,140, PRO140, PRO542, Peptide T, SCH-D, TNX-355, and UK-427,857; treatments for opportunistic infections and other conditions associated with AIDS and HIV including, but not limited to, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, paclitaxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, and valganciclovir; integrase inhibitors including, but not limited to, GS 9137, MK-0518; microbicides, including, but not limited to, BMS-378806, C31G, carbopol 974P, carrageenan, cellulose sulfate, cyanovirin-N, dextran sulfate, hydroxyethyl cellulose, PRO2000, SPL7013, tenofovir, UC-781, and IL-2.

[0079] Additional exemplary combinatorial formulations and coordinate treatment methods may additionally employ the phorbol ester of Formula I in combination with one or more secondary anti-tumor agent(s), or with one or more adjunctive therapeutic agent(s) that is/are useful for treatment or prophylaxis of the targeted (or associated) disease, condition and/or symptom(s) in the selected combinatorial formulation or coordinate treatment regimen. For most combinatorial formulations and coordinate treatment methods of the invention, a phorbol ester compound of Formula I or related or derivative compound is formulated, or coordinately administered, in combination with one or more secondary or adjunctive therapeutic agent(s), to yield a combined formulation or coordinate treatment method that is combinatorially effective or coordinately useful to treat neoplastic diseases and one or more symptom(s) of a secondary disease or condition in the subject. Exemplary combinatorial formulations and coordinate treatment methods in this context employ a phorbol ester compound of Formula I in combination with one or more secondary or adjunctive therapeutic agents selected from, e.g., chemotherapeutic agents, anti-inflammatory agents, doxorubicin, vitamin D3, cytarabine, cytosine arabinoside, daunorubicin, cyclophosphamide, gemtuzumab ozogamicin, idarubicin, mercaptopurine, mitoxantrone, thioguanine, aldesleukin, asparaginase, carboplatin, etoposide phosphate, fludarabine, methotrexate, etoposide, dexamethasone, and choline magnesium tri salicylate. In addition, adjunctive or secondary therapies may be used such as, but not limited to, radiation treatment, hormone therapy and surgery.

[0080] In certain embodiments the invention provides combinatorial cytopathic disease treating (AIDS treating, HIV preventing, HIV treating, HIV reservoir activating, Th1 cytokine increasing, ERK phosphorylation inducing, apoptosis inducing, chemotherapeutic, anti-tumor, cancer treating, remission inducing, remission maintaining) formulations comprising a phorbol ester and one or more adjunctive agent (s) having cytopathic disease treating activity. Within such combinatorial formulations, a phorbol ester of Formula I and the adjunctive agent(s) having cytopathic disease treating activity will be present in a combined formulation in cytopathic disease treating (AIDS treating, HIV preventing, HIV treating, HIV reservoir activating, Th1 cytokine increasing, apoptosis inducing, ERK phosphorylation inducing, chemotherapeutic, anti-tumor, cancer treating, remission inducing, remission maintaining) effective amounts, alone or in combination. In exemplary embodiments, a phorbol ester compound of Formula I and a non-phorbol ester agent(s) will each be present in a cytopathic disease treating amount (i.e., in singular dosage which will alone elicit a detectable alleviation of symptoms in the subject). Alternatively, the combinatorial formulation may comprise one or both the phorbol ester compound of Formula I and the non-phorbol ester agents in sub-therapeutic singular dosage amount(s), wherein the combinatorial formulation comprising both agents features a combined dosage of both agents that is collectively effective in eliciting a cytopathic disease or condition symptom alleviating response. Thus, one or both of the phorbol ester of Formula I and non-phorbol ester agents may be present in the formulation, or administered in a coordinate administration protocol, at a sub-therapeutic dose, but collectively in the formulation or method they elicit a detectable decrease in symptoms of cytopathic disease in the subject. For example, in some embodiments, the combinatorial formulation may include one or more compounds from a highly active antiretroviral therapy protocol (HAART protocols) in combination with a phorbol ester, among other combinations. Other combinatorial formulations may, for example, include a phorbol ester and/or compounds effective in treating the opportunistic infections of AIDS as well as compounds from HAART protocols. In other embodiments, the combinatorial formulation may include one or more additional chemotherapeutic agents.

[0081] To practice coordinate administration methods of the invention, a phorbol ester compound of Formula I may be administered, simultaneously or sequentially, in a coordinate treatment protocol with one or more of the secondary or adjunctive therapeutic agents contemplated herein. Thus, in certain embodiments a compound is administered coordinately with a non-phorbol ester agent, or any other secondary or adjunctive therapeutic agent contemplated herein, using separate formulations or a combinatorial formulation as described above (i.e., comprising both a phorbol ester compound of Formula I or related or derivative compound, and a non-phorbol ester therapeutic agent). This coordinate administration may be done simultaneously or sequentially in either order, and there may be a time period while only one or both (or all) active therapeutic agents individually and/or collectively exert their biological activities.

[0082] In one embodiment, such coordinate treatment methods may, for example, follow or be derived from various highly active antiretroviral therapy protocols (HAART protocols) and include regimens such as, but not limited to, two nucleoside analogue reverse transcriptase inhibitors plus one or more protease inhibitor or non-nucleoside analogue reverse transcriptase inhibitor with a phorbol ester of Formula I, among other combinations. Other coordinate treatment methods may, for example, include a phorbol ester and/or treatments for opportunistic infections as well as compounds from HAART protocols. A distinguishing aspect of all such coordinate treatment methods is that the phorbol ester compound of Formula I exerts at least some activity, which yields a favorable clinical response in conjunction with a complementary AIDS symptom decreasing, or distinct, clinical response provided by the secondary or adjunctive therapeutic agent. Often, the coordinate administration of the phorbol ester compound of Formula I with the secondary or adjunctive therapeutic agent will yield improved therapeutic or prophylactic results in the subject beyond a therapeutic effect elicited by the phorbol ester compound of Formula I, or the

secondary or adjunctive therapeutic agent administered alone. This qualification contemplates both direct effects, as well as indirect effects.

[0083] Within exemplary embodiments, a phorbol ester compound of Formula I will be coordinately administered (simultaneously or sequentially, in combined or separate formulation(s)), with one or more secondary HIV treating agents, or other indicated or adjunctive therapeutic agents, e.g., selected from, for example, protease inhibitors, including, but not limited to, saquinavir, indinavir, ritonavir, nelfinavir, atazanavir, darunavir, fosamprenavir, tipranavir and amprenavir; nucleoside reverse transcriptase inhibitors including but not limited to, zidovudine, didanosine, stavudine, lamivudine, zalcitabine, emtricitabine, tenofovir disoproxil fumarate, AVX754 and abacavir; non-nucleoside reverse transcriptase inhibitors including, but not limited to, nevaripine, delavirdine, calanolide A, TMC125 and efavirenz; combination drugs including, but not limited to, efavirenz/emtricitabine/tenofovir disoproxil fumarate, lamivudine/zidovudine, abacavir/lamivudine, abacavir/lamivudine/zidovudine, emtricitabine/tenofovir disoproxil fumarate, sulfamethoxazole/trimethoprim, and lopinavir/ritonavir; entry and fusion inhibitors, including, but not limited to, enfuvirtide, AMD070, BMS-488043, fozivudine tidoxil, GSK-873,140, PRO140, PRO542, Peptide T, SCH-D, TNX-355, and UK-427,857; treatments for opportunistic infections and other conditions associated with AIDS and HIV including, but not limited to, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, paclitaxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, and valganciclovir; integrase inhibitors including, but not limited to, GS 9137, MK-0518; microbicides, including, but not limited to, BMS-378806, C31G, carbopol 974P, carrageenan, cellulose sulfate, cyanovirin-N, dextran sulfate, hydroxyethyl cellulose, PRO2000, SPL7013, tenofovir, and UC-781, and IL-2.

[0084] In another embodiment, such coordinate treatment methods may, for example, follow or be derived from various chemotherapeutic protocols. Other coordinate treatment methods may, for example, include a phorbol ester and/or treatments for additional symptoms of neoplastic diseases. A distinguishing aspect of all such coordinate treatment methods is that the phorbol ester compound of Formula I exerts at least some activity, which yields a favorable clinical response in conjunction with a complementary neoplastic disease symptom decreasing, or distinct, clinical response provided by the secondary or adjunctive therapeutic agent. Often, the coordinate administration of the phorbol ester compound of Formula I with the secondary or adjunctive therapeutic agent will yield improved therapeutic or prophylactic results in the subject beyond a therapeutic effect elicited by the phorbol ester compound of Formula I, or the secondary or adjunctive therapeutic agent administered alone. This qualification contemplates both direct effects as well as indirect effects.

[0085] Within exemplary embodiments, a phorbol ester compound of Formula I will be coordinately administered (simultaneously or sequentially, in combined or separate formulation(s)), with one or more secondary cancer treating agents, or other indicated or adjunctive therapeutic agents, e.g. doxorubicin, vitamin D3, cytarabine, cytosine arabinoside daunorubicin, cyclophosphamide, gemtuzumab ozogamicin, idarubicin, mercaptopurine, mitoxantrone, thioguanine, aldesleukin, asparaginase, carboplatin, etoposide phosphate, fludarabine, methotrexate, etoposide, dexamethasone, and choline magnesium trisalicylate.

[0086] As noted above, in all of the various embodiments of the invention contemplated herein, the cytopathic disease treating methods and formulations may employ a phorbol ester compound of Formula I in any of a variety of forms, including any one or combination of the subject compound's pharmaceutically acceptable salts, solvates, isomers, enantiomers, polymorphs, solvates, hydrates, and/or prodrugs. In exemplary embodiments of the invention, TPA is employed within the therapeutic formulations and methods for illustrative purposes.

[0087] The pharmaceutical compositions of the present invention may be administered by any means that achieve their intended therapeutic or prophylactic purpose. Suitable routes of administration for the compositions of the invention include, but are not limited to, conventional delivery routes, devices and methods including injectable methods such as, but not limited to, intravenous, intramuscular, intraperitoneal, intraspinal, intrathecal, intracerebroventricular, intraarterial, subcutaneous and intranasal routes.

[0088] The compositions of the present invention may further include a pharmaceutically acceptable carrier appropriate for the particular mode of administration being employed. Dosage forms of the compositions of the present invention include excipients recognized in the art of pharmaceutical compounding as being suitable for the preparation of dosage units as discussed above. Such excipients include, without intended limitation, binders, fillers, lubricants, emulsifiers, suspending agents, sweeteners, flavorings, preservatives, buffers, wetting agents, disintegrants, effervescent agents and other conventional excipients and additives.

[0089] If desired, the compositions of the invention can be administered in a controlled release form by use of a slow release carrier, such as a hydrophilic, slow release polymer. Exemplary controlled release agents in this context include, but are not limited to, hydroxypropyl methyl cellulose, having a viscosity in the range of about 100 cps to about 100,000 cps or other biocompatible matrices such as cholesterol.

[0090] Some phorbol ester compositions of Formula I of the invention are designed for parenteral administration, e.g. to be administered intravenously, intramuscularly, subcutaneously or intraperitoneally, including aqueous and non-aqueous sterile injectable solutions which, like many other contemplated compositions of the invention, may optionally contain anti-oxidants, buffers, bacteriostats and/or solutes which render the formulation isotonic with the blood of the mammalian subject; and aqueous and non-aqueous sterile suspensions which may include suspending agents and/or thickening agents. The formulations may be presented in unit-dose or multi-dose containers. Additional compositions and formulations of the invention may include polymers for extended release following parenteral administration. The parenteral preparations may be solutions, dispersions or emulsions suitable for such administration. The subject agents may also be formulated into polymers for extended release following parenteral administration. Pharmaceutically acceptable formulations and ingredients will typically be sterile or readily sterilizable, biologically inert, and easily administered. Such polymeric materials are well known to those of ordinary skill in the pharmaceutical compounding

arts. Parenteral preparations typically contain buffering agents and preservatives, and injectable fluids that are pharmaceutically and physiologically acceptable such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like. Extemporaneous injection solutions, emulsions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as described herein above, or an appropriate fraction thereof, of the active ingredient(s).

[0091] In more detailed embodiments, compositions of the invention may comprise a phorbol ester compound of Formula I encapsulated for delivery in microcapsules, microparticles, or microspheres, prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly (methylmethacylate) microcapsules, respectively; in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules); or within macroemulsions.

[0092] As noted above, in certain embodiments the methods and compositions of the invention may employ pharmaceutically acceptable salts, e.g., acid addition or base salts of the above-described phorbol ester compounds of Formula I and/or related or derivative compounds. Examples of pharmaceutically acceptable addition salts include inorganic and organic acid addition salts. Suitable acid addition salts are formed from acids which form non-toxic salts, for example, hydrochloride, hydrobromide, hydroiodide, sulphate, hydrogen sulphate, nitrate, phosphate, and hydrogen phosphate salts. Additional pharmaceutically acceptable salts include, but are not limited to, metal salts such as sodium salts, potassium salts, cesium salts and the like; alkaline earth metals such as calcium salts, magnesium salts and the like; organic amine salts such as triethylamine salts, pyridine salts, picoline salts, ethanolamine salts, triethanolamine salts, dicyclohexylamine salts, N,N'-dibenzylethylenediamine salts and the like; organic acid salts such as acetate, citrate, lactate, succinate, tartrate, maleate, fumarate, mandelate, acetate, dichloroacetate, trifluoroacetate, oxalate, and formate salts; sulfonates such as methanesulfonate, benzenesulfonate, and p-toluenesulfonate salts; and amino acid salts such as arginate, asparginate, glutamate, tartrate, and gluconate salts. Suitable base salts are formed from bases that form non-toxic salts, for example aluminum, calcium, lithium, magnesium, potassium, sodium, zinc and diethanolamine salts.

[0093] Other detailed embodiments, the methods and compositions of the invention for employ prodrugs of phorbol esters of Formula I. Prodrugs are considered to be any covalently bonded carriers which release the active parent drug in vivo. Examples of prodrugs useful within the invention include esters or amides with hydroxyalkyl or aminoalkyl as a substituent, and these may be prepared by reacting such compounds as described above with anhydrides such as succinic anhydride.

[0094] The invention disclosed herein will also be understood to encompass methods and compositions comprising phorbol esters of Formula I using in vivo metabolic products of the said compounds (either generated in vivo after administration of the subject precursor compound, or directly administered in the form of the metabolic product itself). Such products may result for example from the oxidation, reduction, hydrolysis, amidation, esterification and the like of the administered compound, primarily due to enzymatic processes. Accordingly, the invention includes methods and compositions of the invention employing compounds produced by a process comprising contacting a phorbol ester compound of Formula I with a mammalian subject for a period of time sufficient to yield a metabolic product thereof. Such products typically are identified by preparing a radiolabelled compound of the invention, administering it parenterally in a detectable dose to an animal such as rat, mouse, guinea pig, monkey, or to man, allowing sufficient time for metabolism to occur and isolating its conversion products from the urine, blood or other biological samples.

[0095] The invention disclosed herein will also be understood to encompass diagnostic compositions for diagnosing the risk level, presence, severity, or treatment indicia of, or otherwise managing cytopathic diseases including, but not limited to, neoplastic disesases including malignant neoplastic diseases such as leukemia, and an AIDS or a related disease or condition in a mammalian subject, comprising contacting a labeled (e.g., isotopically labeled, fluorescent labeled or otherwise labeled to permit detection of the labeled compound using conventional methods) phorbol ester compound of Formula I to a mammalian subject (e.g., to a cell, tissue, organ, or individual) at risk or presenting with one or more symptom(s) of cancer and/or AIDS, and thereafter detecting the presence, location, metabolism, and/or binding state (e.g., detecting binding to an unlabeled binding partner involved in HIV receptor physiology/metabolism or malignant cell receptor physiology/metabolism) of the labeled compound using any of a broad array of known assays and labeling/detection methods. In exemplary embodiments, a phorbol ester compound of Formula I is isotopically-labelled by having one or more atoms replaced by an atom having a different atomic mass or mass number. Examples of isotopes that can be incorporated into the disclosed compounds include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, and ^{36}Cl, respectively. The isotopically-labeled compound is then administered to an individual or other subject and subsequently detected as described above, yielding useful diagnostic and/or therapeutic management data, according to conventional techniques.

EXAMPLES

[0096] The experiments described below demonstrate novel and powerful uses for phorbol esters and derivative compounds as HIV treating drugs that can effectively decrease the symptoms of AIDS. In exemplary clinical trials, individuals who were unresponsive to traditional treatments for HIV and AIDS were responsive to treatments with TPA. The treatment with TPA was allowed as "compassionate" and recovery of some patients was considered life-saving according to the attending physicians. The experiments described below additionally demonstrate the usefulness of phorbol esters and derivative compounds in the treatment of neoplastic diseases. These and additional findings are further expanded and elucidated within the following examples.

Example I

Effect of TPA on the Peripheral White Blood Cells (WBC) and Hemoglobin (Hb) Counts in 5180 Cell-Injected Mice:

[0097] Sarcoma 180 (S180) cells were injected into Kwen-Ming mice. On the third day, the mice were given TPA inter-

peritoneally (i.p.). at 50, 100 or 200 μg/kg/day for 7 days. On the second day after the treatment was completed, blood samples were taken from the tails of the treated mice for WBC and Hb analyses. The WBC counts for the treated groups (50, 100, or 200 ug/kg/day for 7 days) were 16.1±7.4, 18.7±.3.0 and 20.7±.3.4×10^9/L, respectively; the WBC count for the control group was 13.6±1.8×10^9/L. The Hb of the treated groups were 136±11, 149±12 and 149±10 g/L, and the Hb of the control group was 134+-15 g/L. The results indicate that i.p. injection of TPA could increase the peripheral WBC counts in mice in a dose-dependent manner, whereas the Hb levels were not greatly affected in TPA treated mice when compared to the control mice.

Example II

Dose Ranging Study

[0098] Due to the strong local irritation caused by TPA application. TPA was given to patients by intravenous (i.v.) infusion. TPA solution in a sterile syringe was injected into 200 ml of sterile saline and mixed well for i.v. infusion.

[0099] The Toxicity and Side Effects of Different TPA Doses Administered Clinically:

[0100] (1) TPA Given at 1 mg/Patient/Week:

[0101] One mg TPA in solution was mixed well with 200 ml of sterile saline for intravenous infusion which was completed in 1 h at the rate of 16 μg/min. One hour after TPA administration, patients started to have chills which lasted for about 30 min, followed by fever, (the patients' temperature reached 37.5-39.5° C. which lasted for 3-5 h, then returned to normal) with light to heavy perspiration. The above symptoms could be alleviated by giving the patients glucocorticoids. TPA at this dose caused a minority of patients to bleed, several patients suffered for a short period of time difficulty in breathing, and Hb was detected in the urine. However, these side effects were short lived and reversible. The cardiac, hepatic, renal and pulmonary functions were all found to be normal.

[0102] (2) TPA Given at 0.5 mg/Patient×2/Week: (Two Doses a Week)

[0103] 0.5 mg of TPA in solution was mixed well with 200 ml of saline for intravenous infusion which was completed in 1 h at the rate of 8 mg/min. The reactions after administration were similar to that of the 1 mg TPA dosage, but to a lesser extent than the 1 mg dose. The patients tolerated the lower dose more easily. Occasionally, Hb was detected in patients' urine. Difficulty in breathing was not observed. The cardiac, hepatic, renal and pulmonary functions were all normal.

[0104] (3) TPA Given at 0.25 mg/Patient×4/Week:

[0105] 0.25 mg of TPA in solution was mixed well with 200 ml of saline for intravenous infusion which was completed in 1 h at the rate of 4 μg/min. After administration, symptoms such as chills and fever were also observed, but to a much lesser extent than with the higher dosages. No Hb was detected in the urine, and no patient suffered difficulty in breathing. The cardiac, hepatic, renal and pulmonary functions were all normal.

Example III

First Clinical Study of HIV+Patients Treated with TPA

[0106] Twelve symptomatic patients (five males and seven females) between the ages of 35 to 52 all of whom were infected with HIV in 1995 through blood transfusion and were refractory to standard treatments for HIV were treated with TPA. Each patient was administered a weight adjusted dosage of TPA (75 μg/sq m) in 200 ml of sterile saline by i.v. over one hour. This dose was administered once daily for the first three days of treatment. Each patient was then given this dose every other day for days 4 to 18 followed by a six month rest period prior to a second course of treatment according to the same protocol.

[0107] Blood samples were gathered prior to administration of the first dose of TPA and on days 4 and 40 of the treatment cycle. Levels of CD3, CD4 and CD8 in peripheral blood were measured using monoclonal antibodies (Becton Dickson Scientific Co., Franklin Lakes, N.J.) and a flow cytometer (B.D. Bioscience, San Diego, Calif.).

[0108] As can be seen in Table 1, no consistent change or correlation was observed in CD3, CD4, or CD8 levels.

TABLE ONE

CD_4 CD_8 CD_3 TEST RESULTS OF TWELVE HIV PATIENTS

PATIENT NO	TEST TIME	CD_4	CD_8	CD_3
01-1	Before TPA	3	196	341
01-2	Four days after TPA	3	180	299
01-3	Forty two days after TPA	2	111	203
02-1	Before TPA	26	614	687
02-2	Four days after TPA	105	<2000	2616
02-3	Forty two days after TPA	54	700	799
03-1	Before TPA	32	524	543
03-2	Four days after TPA	36	366	427
03-3	Forty two days after TPA	33	374	424
04-1	Before TPA	173	735	975
04-2	Four days after TPA	123	770	941
04-3	Forty two days after TPA	44	493	581
05-1	Before TPA	106	1556	1646
05-2	Four days after TPA	119	1330	1282
05-3	Forty two days after TPA	191	1429	1643
06-1	Before TPA	232	865	1221
06-2	Four days after TPA	179	570	808
06-3	Forty two days after TPA	49	429	537
07-1	Before TPA	10	988	1022
07-2	Four days after TPA	7	570	598
07-3	Forty two days after TPA	1	139	146
08-1	Before TPA	524	725	1332
08-2	Four days after TPA	318	355	739
08-3	Forty two days after TPA	241	527	858
09-1	Before TPA	442	1021	1479
09-2	After TPA	663	<2000	2920
10-1	Before TPA	407	328	778
10-2	After TPA	445	591	1077
11-1	Before TPA	40	322	373
11-2	After TPA	131	724	874
12-1	Before TPA	84	256	375
12-2	After TPA	78	268	362

[0109] As can be seen in Table 2, below, there were similarly inconsistent results in the change of viral load with five patients having an increase in HIV and no change or a reduction in seven others.

TABLE TWO

BLOOD HIV COUNT OF THE TWELVE PATIENTS BEFORE DURING AND AFTER THE TPA TREATMENT

PATIENT NO	TEST TIME	RESULTS (copies/ml)	LOG VALUE	FOOT NOTE
01-1	3 days before TPA	3.36×10^5	5.526	
01-2	4 days after initial TPA	1.41×10^4	6.151	
01-3	15 days after initial TPA	2.02×10^4	4.306	
01-4	25 days after initial TPA	2.60×10^4	4.416	
02-1	3 days before TPA	9.97×10^4	4.999	
02-2	4 days after initial TPA	7.92×10^6	6.899	
02-3	15 days after initial TPA	6.33×10^6	6.801	
02-4	25 days after initial TPA	8.72×10^6	6.941	
03-1	3 days before TPA	3.77×10^5	5.577	
03-2	4 days after initial TPA	8.13×10^4	4.910	
03-3	15 days after initial TPA	6.11×10^3	3.786	
03-4	25 days after initial TPA	8.59×10^5	5.934	
04-1	3 days before TPA	1.11×10^6	6.045	
04-2	4 days after initial TPA	1.75×10^7	7.243	
04-3	15 days after initial TPA	1.11×10^6	6.614	
04-4	25 days after initial TPA	1.21×10^4	4.084	
05-1	3 days before TPA	2.49×10^4	6.637	
05-2	4 days after initial TPA	9.42×10^5	5.974	
05-3	15 days after initial TPA	2.34×10^7	7.369	
05-4	25 days after initial TPA	5.56×10^6	6.745	
06-1	3 days before TPA	4.57×10^5	5.660	
06-2	4 days after initial TPA	1.44×10^4	4.160	
06-3	15 days after initial TPA	1.88×10^5	5.274	
06-4	7 days after TPA	2.28×10^6	6.357	
07-1	3 days before TPA	2.40×10^5	5.623	
07-2	4 days after initial TPA	1.51×10^5	5.179	
07-3	15th day during TPA	9.74×10^4	4.988	
07-4	25 days after initial TPA	5.30×10^3	3.724	
08-1	3 days before TPA	8.02×10^5	5.904	
08-2	4 days after initial TPA	9.09×10^5	5.959	
08-3	15 days after initial TPA	5.46×10^6	6.737	
08-4	25 days after initial TPA	7.77×10^6	6.890	
09-1	3 days before TPA	undetectable		
09-2	25 days after TPA	undetectable		
10-1	3 days before TPA	1.51×10^4	4.180	Sample taken from the
10-2	25 days after initial TPA	2.79×10^4	4.446	second cycle treatment
11-1	3 days before TPA	1.59×10^5	5.201	Sample taken from the
11-2	25 days after initial TPA	1.25×10^5	5.096	second cycle treatment
12-1	3 days before TPA	1.32×10^4	4.122	Sample taken from the
12-2	25 days after initial TPA	6.27×10^3	3.798	second cycle treatment

[0110] Despite the lack of correlation with viral and CD3, CD4 and CD9 levels, eleven of the patients showed significant improvement following treatment. Eight patients became symptom free and five of them have been in remission for 6 to 12 months. Three additional patients had a decrease in symptoms.

Example IV

Second Clinical Study of HIV+Patients Treated with TPA

[0111] Nine of the patients in Example III were given a second treatment of TPA. Of these nine, seven were asymptomatic at the beginning of the second trial. A tenth patient (patient #2a) who was symptomatic and had not previously been treated with TPA was added to the study. Each patient was administered a weight adjusted dosage of TPA (75 μg/sq m) in 200 ml of sterile saline intravenously over one hour. This dosage was given to each patient once a day for ten consecutive days followed by a rest period of ten days for three cycles and a total of 30 doses of TPA. Patients 5a, 6a, and 8a stopped taking anti-AIDS drugs one month prior to beginning the TPA treatment and beginning again one month after the third cycle. Patients 1-4-a, 7a, and 9a-10a continued taking anti-AIDS drugs throughout the treatment.

[0112] Blood samples were taken three days prior to starting treatment, after completing the first 10 day cycle of TPA infusion and again after the last TPA infusion and CD3, CD4, CD8, WBC, RBC, HGB and platelets were measured.

[0113] As shown in Table 3, there was an increase in CD3 in all patients after the first and third infusion with TPA with the highest value occurring after the third cycle, with the exception of two patients (5a & 10a). There was a trend for increases in the CD8 and in CD4. These results suggest a strengthening of the immune systems with TPA treatment. Varied results were obtained in the HIV count (Table 4). The HIV measurements in some of the patients were below the limits of detection of the method (less than 200) while it increased somewhat in others. There was normal variation in the measurement of WBC, RBC, HGB and platelets (Table 5).

TABLE THREE

CD_4 CD_8 CD_3 TEST RESULTS OF 10 HIV PATIENTS

PATIENT NO	TEST TIME	CD_4	CD_8	CD_3
01-1	Before TPA	5	576	1071
01-2	After first 10-day TPA infusion cycle	7	907	1323
01-3	After third 10-day TPA infusion cycle	19	1129	2037
02a-1	Before TPA	26	307	339
02a-2	After first 10-day TPA infusion cycle	76	335	476
02a-3	After third 10-day TPA infusion cycle	137	543	625
03a-1	Before TPA	295	571	870
03a-2	After first 10-day TPA infusion cycle	460	729	1200
03a-3	After third 10-day TPA infusion cycle	1002	980	2033
04a-1	Before TPA	152	672	896
04a-2	After first 10-day TPA infusion cycle	189	584	823
04a-3	After third 10-day TPA infusion cycle	205	916	1193
05a-1	Before TPA	92	1097	1175
05a-2	After first 10-day TPA infusion cycle	91	1507	1598
05a-3	After third 10-day TPA infusion cycle	94	1127	1257
06a-1	Before TPA	230	378	669
06a-2	After first 10-day TPA infusion cycle	285	429	758
06a-3	After third 10-day TPA infusion cycle	276	466	938
07a-1	Before TPA	567	1736	2258
07a-2	After first 10-day TPA infusion cycle	729	>2000	3148
07a-3	After third 10-day TPA infusion cycle	786	>2000	3347
08a-1	Before TPA	361	569	1023
08a-2	After first 10-day TPA infusion cycle	519	547	1143
08a-3	After third 10-day TPA infusion cycle	495	733	1295
09a-1	Before TPA	101	533	672
09a-2	After first 10-day TPA infusion cycle	136	574	712
09a-3	After third 10-day TPA infusion cycle	100	1221	1317
10a-1	Before TPA	49	178	240
10a-2	After first 10-day TPA infusion cycle	74	261	333
10a-3	After third 10-day TPA infusion cycle	63	208	308

TABLE FOUR

BLOOD HIV COUNT OF THE TEN PATIENTS BEFORE DURING AND AFTER THE THREE TEN-DAY TPA INFUSION

PATIENT NO	TEST TIME	RESULTS (copies/ml)	LOG VALUE
01-1	3 days before TPA	4.57×10^6	6.660
01-2	after first cycle TPA infusion	2.99×10^5	5.475
01-3	after third cycle TPA infusion	9.41×10^5	5.973
02a-1	3 days before TPA	2.71×10^5	5.433
02a-2	after first cycle TPA infusion	3.09×10^5	5.490
02a-3	after third cycle TPA infusion	9.24×10^5	5.966
03a-1	3 days before TPA	undetectable	—
03a-2	after first cycle TPA infusion	lower the 500	2.371
03a-3	after third cycle TPA infusion	9.55×10^3	3.980
04a-1	3 days before TPA	lower than 500	2.312
04a-2	after first cycle TPA infusion	undetectable	—
04a-3	after third cycle TPA infusion	2.38×10^3	3.376
05a-1	3 days before TPA	undetectable	—
05a-2	after first cycle TPA infusion	undetectable	—
05a-3	after third cycle TPA infusion	undetectable	—
06a-1	3 days before TPA	undetectable	—
06a-2	after first cycle TPA infusion	undetectable	—
06a-3	after third cycle TPA infusion	undetectable	—
07a-1	3 days before TPA	undetectable	—
07a-2	after first cycle TPA infusion	undetectable	—
07a-3	after third cycle TPA infusion	undetectable	—
08a-1	3 days before TPA	1.13×10^4	4.054
08a-2	after first cycle TPA infusion	6.68×10^4	4.825
08a-3	after third cycle TPA infusion	6.20×10^4	4.792
09a-1	3 days before TPA	1.38×10^5	5.139
09a-2	after first cycle TPA infusion	1.65×10^5	5.217
09a-3	after third cycle TPA infusion	2.35×10^5	5.371
10a-1	3 days before TPA	7.20×10^5	5.857
10a-2	after first cycle TPA infusion	2.82×10^5	5.450
10a-3	after third cycle TPA infusion	1.86×10^5	5.270

TABLE FIVE

PERIPHERY BLOOD COUNT OF THE TEN PATIENTS BEFORE AND AFTER THE TPA THREE 10-DAY TREATMENT

PATIENT NO	TEST TIME	WBC ($\times 10^9$/L)	RBC ($\times 10^{12}$/L)	HGB (g/L)	PLt ($\times 10^9$/L)
01-1	Before TPA	2.3	2.55	92	199
01-2	After first 10-day TPA infusing	4.4	2.61	99	325
01-3	After third 10-day TPA infusing	6.1	2.91	102	182
02a-1	Before TPA	5.7	2.44	114	227
02a-2	After first 10-day TPA infusing	3.7	2.14	88	238
02a-3	After third 10-day TPA infusing	11.1	2.52	100	124
03a-1	Before TPA	7.8	4.04	147	309
03a-2	After first 10-day TPA infusing	9.8	3.83	1.38	338
03a-3	After third 10-day TPA infusing	13.6	4.54	140	549
04a-1	Before TPA	3.9	3.34	127	232
04a-2	After first 10-day TPA infusing	3.6	2.92	107	306
04a-3	After third 10-day TPA infusing	9.2	2.85	105	105
05a-1	Before TPA	5.1	3.54	146	243
05a-2	After first 10-day TPA infusing	5.7	3.46	1.35	315
05a-3	After third 10-day TPA infusing	10.1	3.61	144	130
06a-1	Before TPA	5.0	4.21	171	198
06a-2	After first 10-day TPA infusing	4.2	3.48	142	256
06a-3	After third 10-day TPA infusing	6.5	3.66	154	169
07a-1	Before TPA	6.6	3.62	102	306
07a-2	After first 10-day TPA infusing	6.0	3.76	143	258
07a-3	After third 10-day TPA infusing	6.0	3.92	123	293
08a-1	Before TPA	3.1	4.03	125	116
08a-2	After first 10-day TPA infusing	4.3	3.86	128	221
08a-3	After third 10-day TPA infusing	6.8	4.19	128	138
09a-1	Before TPA	3.5	1.43	41	114
09a-2	After first 10-day TPA infusing	2.6	1.99	57	214
09a-3	After third 10-day TPA infusing	4.0	2.33	67	170
10a-1	Before TPA	2.6	2.65	78	297

TABLE FIVE-continued

PERIPHERY BLOOD COUNT OF THE TEN PATIENTS BEFORE AND AFTER THE TPA THREE 10-DAY TREATMENT

PATIENT NO	TEST TIME	WBC ($\times 10^9$/L)	RBC ($\times 10^{12}$/L)	HGB (g/L)	PLt ($\times 10^9$/L)
10a-2	After first 10-day TPA infusing	2.9	2.58	92	187
10a-3	After third 10-day TPA infusing	7.0	4.31	130	138

[0114] Of nine patients previously treated with TPA in the first clinical study, only one (#9a) presented with some AIDS symptoms prior to the start of the second clinical study. Following treatment with three cycles of TPA in the second study, this patient and another (#2a), who had never been treated with TPA, experienced a disappearance of AIDS symptoms and both became sufficiently well to resume their normal activities. The other eight patients began the study without AIDS symptoms and were symptom free at the end of the study. All patients remain under observation. Treatment with anti-AIDS drugs continues uninterrupted.

[0115] As can be seen in Table 4, there was an increase in all patients in the CD 3, 4 and 8 levels with the most striking and consistent increases in CD3 levels. The viral load of HIV varied. It was undetectable in three patients (<200); it increased somewhat in six others and was reduced in one.

Example V

Third Clinical Study of HIV+Patients Treated with TPA

[0116] Six patients, two males and four females between the ages of 37 and 52 years of age (Patients #13-18), were treated with TPA. Four of these patients previously received TPA treatment in combination with anti-HIV drugs in the two previous clinical studies. The two remaining patients had never been treated with TPA, but had previously received anti-HIV drug regimens. All treatments were stopped three days prior to the initiation of the third clinical study and were not resumed until 60 days after completion of the TPA treatment. The resumption of the standard HIV treatments was required by local health authorities.

[0117] Each patient in the study received 150 μg of TPA in 200 ml of sterile saline by intravenous infusion over a 1.5 to 2 hour period daily for 60 days for a total administered dose of 9.0 mg. Following completion of the 60 days of TPA therapy, these patients remained under observation for an additional 60 days though the received no further treatment.

[0118] CD3, CD4 and CD8 levels in peripheral blood were quantitated prior to starting treatment, and again at 30 and 60 days using flow cytometry and the appropriate antibodies obtained from B.D. Bioscience, San Diego, Calif. Viral load was determined using conventional methods at Kuang Ann men Hospital, Beijing, China. Patients RBC, WBC, platelets and hemoglobin levels were also measured.

[0119] As can be seen in Table 6, the viral load in the six patients was either low or undetectable at the beginning of the trial and remained low throughout the clinical trial period despite the discontinuation of traditional antiretroviral therapy. Additionally, there was no rebound in viral levels 6 to 15 days after stopping antiretroviral treatment as previously reported as occurring in patients with a plasma viral load below 50 HIV copies per ml. (Harrigan et al., AIDS 13, F59-F62 (1999). The CD3, CD4 and CD8 levels were variable and inconclusive.

TABLE SIX

STUDY 3
$CD_4CD_8CD_3$ AND HIV LOAD RESULTS OF 6 PATIENTS

PATIENT#	*TEST TIME	CD3	CD4	CD8	**HIV (copies/ml)
13	1	3500	1135	>2000	undetectable
	2	2771	735	1938	0.533
	3	2689	721	1897	0.133
14	1	1415	677	664	0.374
	2	1522	613	796	0.353
	3	902	369	485	0.038
15	1	759	9	542	0.533
	2	1865	8	1408	1.99
	3	2099	11	1507	undetectable
16	1	1368	128	1166	undetectable
	2	1477	105	1318	1.28
	3	1305	46	1220	0.012
17	1	428	95	297	0.002
	2	594	112	424	0.152
	3	317	31	246	0.056
18	1	1041	392	457	undetectable
	2	703	229	343	0.174
	3	579	165	290	undetectable

*Test time:
1. Before TPA
2. Thirty days after TPA
3. Sixty days after TPA
**All figures are in the million

[0120] White blood cells (WBC), red blood cells (RBC), hemoglobin (Rb) and platelets (PLt) were measured prior to starting TPA treatment, 15, 30, 45 and 60 days after starting TPA treatment and 30 days after stopping TPA treatment. As can be seen in Table 7, most values were within the normal range.

[0121] The patients involved in the third clinical study experienced no viral load rebound as typically seen when antiretroviral therapies are discontinued. They additionally had no recurrence of AIDS symptoms during the 120 day observation and treatment period, felt normal and were able to conduct their usual life activities.

TABLE SEVEN

STUDY 3
PERIPHERY BLOOD PROFILE OF 6 PATIENTS

PATIENT#	*TEST TIME	WBC ($\times 10^9$/L) ×	RBC ($\times 10^{12}$/L)	Rb (g/L)	PLt ($\times 10^9$/L)
13	1	9	3.75	139	246
	2	9	3.88	140	240

TABLE SEVEN-continued

STUDY 3
PERIPHERY BLOOD PROFILE OF 6 PATIENTS

PATIENT#	*TEST TIME	WBC ($\times 10^9$/L) ×	RBC ($\times 10^{12}$/L)	Rb (g/L)	PLt ($\times 10^9$/L)
	3	8.9	4.35	148	275
	4	4.6	3.9	125	304
	5	8.8	4.55	126	221
	6	7.5	4.55	130	272
14	1	4.2	4.16	111	188
	2	4.1	4.03	114	169
	3	5.9	4.48	116	232
	4	3.9	4.44	109	152
	5	4.4	4.31	96	227
	6	6.5	4.4	104	193
15	1	5.9	3.67	110	397
	2	5	3.41	101	219
	3	5.2	3.83	113	247
	4	6.2	4.13	110	262
	5	6.2	4.04	99	239
	6	8.4	3.9	110	278
16	1	6	3.62	144	297
	2	8.1	3.65	142	415
	3	4.3	4.03	145	345
	4	4.6	3.86	124	291
	5	5.1	4.1	123	276
	6	3.8	4.71	144	224
17	1	5.5	3.06	124	242
	2	6.4	2.98	118	151
	3	4	3.2	121	177
	4	3.9	3.49	116	131
	5	7.7	3.34	99	121
	6	4.8	3.42	100	178
18	1	7.4	3.91	156	240
	2	8.1	3.69	141	208
	3	4.5	4.32	154	228
	4	4.9	4.14	131	149
	5	3.5	4.56	136	222
	6	NA	NA	NA	NA

*Test time:
1. Before TPA
2. Fifteen days after TPA
3. Thirty days after TPA
4. Forty five days after TPA
5. Sixty day after TPA
6. Thirty days after stop TPA

Example VI

Case Studies

[0122] Results of treatment of initially symptomatic AIDS patients treated with TPA according to the protocols of Example III, IV, and V. Patients who participated in multiple studies are in some cases identified by more than one patient number. All patient identification numbers correspond to the patient numbers in Tables 1-7.

[0123] Patient #1 and 15: H.L.Y., female, 35, participated in all three clinical studies, diagnosed with AIDS and had clear symptoms of this disease in 2003. At the time the first study began, she had frequent fever, diarrhea, oral lesions, poor appetite, weight loss, left eye vision loss (syncytia formation) and coughing (tuberculosis). The patient started to receive antiviral medications Stavudine (D_4T), Lamivudine (3TC), Nevirapine (NVP) and Zidovudine (AZT) in 2004. Despite anti-AIDS drugs, she had a CD4 count of 3 and was unable to perform any physical work.

[0124] During the first study following the protocol of Example III, above, she experienced an increase in body temperature of 38-39° C. on four different occasions that lasted 2 to 4 hours. After treatment with TPA, there was a gradual improvement in symptoms. Her appetite improved and diarrhea, oral lesions, and fatigue disappeared but her eyesight remained impaired. She gained some weight and reported being able to resume housework. She continues to receive antiviral therapy. There appears to be no correlation in improvements in symptoms and changes in her CD 3, 4, 8 levels and viral count.

[0125] H. L. Y. participated in the second study described in Example IV, above. At the initiation of the second study she has no symptoms of AIDS. During this subsequent treatment with TPA she experienced no adverse effects. After both the first and third cycle of treatment with TPA, her CD3, CD4, and CD8 levels increased as did her white blood cell count. Her HIV count was somewhat higher, but she is able to function normally and continues to have no symptoms of AIDS.

[0126] H.L.Y. participated in the third study described in Example V, above. At the initiation of the third study, she was still having problems with her eye. During the third study, she experienced a fever of 38-38.5° C. during the third and fourth day of TPA infusion. No AIDS symptoms returned during either the study or the 60 day observation period. Except for her sight, she remains symptom free, feels normal and is able to conduct normal activities. She reinitiated antiviral therapy after completion of the 60 day observation period and remains under the care of a physician.

[0127] Patient #2: C.X., female, 49, participated in first clinical study, diagnosed with AIDS and had clear symptoms of this disease in 2004. She had mild oral lesions, fatigue, skin thrush, fever and poor appetite. Some of these symptoms were due to herpes virus. She had been treated with AZT, DDI and NVP but drug treatment was terminated due to side effects. She received no drugs for 3 months prior to TPA treatment. She was hospitalized frequently and was unable to work. Her CD4 count prior to treatment was 26.

[0128] During TPA treatment according to the protocol of Example III, she experienced an increase in body temperature of 37.5 to 38 degrees centigrade on three different occasions that lasted 1-2 hours. After treatment with TPA, her oral lesions, skin thrush and fever disappeared. Her appetite improved sufficiently so that she gained weight and had sufficient energy to resume housework. She remained symptom free for five months and was not given any anti-AIDS drugs during this period. There appeared to be no correlation between the improvement in symptoms and her CD 3, 4, 8 levels and viral count.

[0129] Patient #2a M. S., male, 48, participated only in the second clinical study, had frequent fever, diarrhea, weight loss, a weak immune system, severe depression and was unable to work.

[0130] During treatment with TPA according to the protocols of Example IV, his body temperature increased to 38.5 to 39 degrees centigrade on five occasions for 2 to 4 hours.

[0131] After the third cycle of TPA treatment, the fever and diarrhea were no longer a problem. His CD3, CD4 and CD8 counts trended upwards as did the WBC and HIV count. His physical and mental condition returned to normal and he is able to work. Patient #3: Y.P., male, 51, participated only in the first clinical study, diagnosed with AIDS and had clear symptoms of this disease in 2004. His major symptoms were diarrhea, fatigue, weight loss, anemia and purple marks on the skin of both legs; and he could only do light work. He was

being treated with AZT, DDI and NVP but a serious anemia resulted in the termination of drug treatment four months prior to being given TPA. His initial CD4 count was 32.

[0132] During TPA treatment according to the protocol described in Example III, he experienced an increase in body temperature of 38 to 39° C. on three occasions that lasted 1 to 2 hours. After treatment with TPA, there was a marked improvement in his symptoms and he was able to return to work involving heavy labor and is leading a normal life. He was symptom free for five months after TPA therapy and was not treated with antiviral drugs during this period. There appeared to be no correlation between CD 3, 4, and 8 levels and improvement in symptoms but there was some increase in viral count.

[0133] Patient #4: L.W., male, 34, participated in only the first clinical study, tested positive for HIV and had clear symptoms of this disease in 2004. His major symptoms were diarrhea, fever, weight loss, cough (tuberculosis), right side neck lymph node enlargement and he was unable to work. His initial response to treatment was poor. The schedule of antiviral medication of 3TC, DDI and NVP was irregular and was stopped during TPA therapy. His initial CD4 count was 173.

[0134] During treatment with TPA according to the protocol of Example III, he experienced an increase in body temperature of 38 to 39° C. on five occasions that lasted 0.5 to 1 hours. After treatment. the occasional bout of diarrhea was treated successfully with and an anti-diarrhea drug. An improvement in appetite has resulted in an increase in weight and energy that resulted in his returning to a regular work schedule. The lymph node returned to normal size. He continues to be treated with anti-viral drugs. There appeared to be no correlation between the improvements in symptoms, CD3, 4, 8 levels and viral count.

[0135] Patient #5 and 3a: H.S., female, 37, participated in the first two clinical studies, tested positive for HIV and had clear symptoms of the disease in of 2004. At the time the first study began, her major symptoms were skin thrush, hair loss, mouth infection, weight loss and fatigue. She was being treated with D_4T, DDI, and NVP but treatment was stopped due to loss of kidney function. She had an initial CD4 count of 106 but could handle regular labor work.

[0136] During treatment with TPA according to the protocol of Example III, she experienced in increase in body temperature of 37.5 to 38° C. on five occasions that lasted 0.5 to 1.0 hours. After treatment with TPA, no improvement in symptoms occurred. Treatment with anti-viral drugs was resumed without return of the previous side effects and the intensity of her symptoms were reduced after one month. This treatment is being continued and she has returned to work. There appeared to be no correlation between the improvement in symptoms and changes in the CD 3, 4, and 8 levels or the HIV count.

[0137] At the time of the second study, she had no symptoms of AIDS and suffered no adverse effects to the course of treatment described in Example IV. After the second study, her CD3, CD4 and CD8 levels trended upwards as did her white blood count and platelet levels. Her HIV count was initially undetectable, but increased after the third cycle of treatment. She is currently able to work.

[0138] Patient #6, #4a, and #17: H.S.C., male, 36, participated in all three clinical studies, tested positive for HIV and had clear but mild symptoms in 2004. At the time the first study began, he suffered from dizziness, headache, poor appetite and an increased susceptibility to upper respiratory tract infections but was able to work regularly as a laborer. He was being treated with antiviral drugs AZT, DDI and NVP but terminated their use due to adverse reactions. His initial CD4 level was 232.

[0139] During treatment with TPA according to the protocol of Example III, he did not experience an increase in body temperature or any other side effect. After treatment, his symptoms remained unchanged and a reduction in platelets appeared unrelated to TPA treatment. He continued to be treated with antiviral drugs and is able to work as before. There appeared to be no correlation between the improvement in symptoms and the CD 3, 4, and 8 levels and viral load.

[0140] At the time of the second study, he had no symptoms and his immune system appeared to be functioning normally. During the second study according to Example IV, he again suffered no side effects from treatment with TPA. His CD3, CD4, and CD8 count increased somewhat as did his white blood cell count. The viral load was initially undetectable but increased after the third cycle of treatment. However, he does not have any symptoms of AIDS and has returned to work.

[0141] At the initiation of the third clinical study, he had no symptoms. During treatment with TPA according to the protocol of Example V, he experienced an incident of local irritation due to a leaking needle on day 32 but was treated successfully in three days. He remains symptom free, feels normal, and is able to do heavy labor. He started antiviral therapy after completion fo the 60 day observation periods and remains under the care of a physician.

[0142] Patient #7, #5a and #16: H. C. L., male, 49, participated in all three clinical studies, tested positive for HIV and had clear symptoms of the disease in 2004. His major symptoms at the time of the first study were weight loss, skin thrush, fatigue, poor appetite and coughing (tuberculosis) but he was able to do light work. He was treated simultaneously with D_4T, DDI, NVP and antituberculosis medication. His initial CD4 count was 10.

[0143] During treatment with TPA according to the protocol outlined in Example III, he experienced an increase in body temperature to 38° C. on two occasions accompanied by mild dizziness and headache. After treatment, his symptoms remained unchanged and antiviral therapy was resumed one month later. With time, his cough, appetite and energy level improved and he is able to work. He continued both antiviral and anti-tuberculosis medication. There appeared to be no correlation between improvements in symptoms and his CD3, 4, and 8 levels or viral load.

[0144] At the time of the second clinical investigation, he had no symptoms of AIDS and his immune system appeared to be functioning normally. He suffered no adverse effects from treatment TPA during the second clinical investigation. After treatment, his CD4 level was unchanged, but his CD3 and CD8 levels trended upwards as did his white blood cell count. His viral load was undetectable. He has not had any symptoms of AIDS and has returned to work.

[0145] At the start of the third clinical investigation, he was not experiencing AIDS symptoms. During treatment according to the protocol outlined in Example V, he suffered from a fever on one occasion. He remains symptom free, feels normal, and is able to do heavy labor. He re-started antiviral drugs after completion of the 60 day observation period and remains under the care of a physician.

[0146] Patient #8, #6a, and 18: Y.X.O., female, 36, participated in all three clinical studies, tested positive for HIV in 2004. Her major symptom at the time of the first study was an

increased susceptibility to upper respiratory tract infection. She was treated with AZT, DDI and NVP. At the start of the study, her CD4 level was 524 and she could handle regular labor work.

[0147] During treatment with TPA according to the protocol of Example III, she experienced an increase in body temperature to 38.5° C. on one occasion that lasted four hours. After treatment, the frequency of her colds decreased and she had no other symptoms. She continued to be treated with antiviral drugs and is able to work. There appeared to be no correlation between the improvement in symptoms and her CD 3, 4, or 8 levels or viral load.

[0148] At the time of the second clinical investigation, she had no symptoms of AIDS and her immune system appeared to be functioning normally. During the second study, according to the protocols of Example IV, her body temperature again rose to 38.5 degrees centigrade for two hours on a single occasion. After treatment, her CD3 and CD8 levels increased somewhat while her CD4 and white blood cell count remained unchanged. Her viral load is undetectable. She appears normal and is able to work at physically demanding tasks.

[0149] At the time of the third clinical investigation she was symptom free. The only side effects from treatment according to the protocol of Example V was as fever of 38-39° C. on the second day of the treatment that lasted for two hours and skin irritation from a leaking needle on day 36 that cleared in two days. She remains symptom free, feels normal and is able to do heavy labor. She re-started antiviral therapy after completion of the 60 day observation period and remains under the care of a physician.

[0150] Patient #9 and #7a: C.T.F., male, 44, participated in the first two clinical studies, tested positive for HIV and had clear symptoms of the disease in 2004. His symptoms at the initiation of the first study included persistent diarrhea, dizziness, headaches, poor appetite, weight loss and fatigue. He had a positive response to AZT, DDI and NVP treatment and blood HIV count was near the lowest limit. Despite the positive response, his symptoms persisted and he checked into the hospital due to diarrhea that persisted for 20 days. He was very depressed and unable to do any work.

[0151] During treatment with TPA according to the protocol of Example III, he experienced an increase in body temperature of 37.5 to 38° C. on six occasions that lasted 2 to 4 hours. A leaking needle caused a serious skin irritation during one administration of TPA but was treated successfully. After eight treatments with TPA, the mild dizziness and headache persisted but the incidence of diarrhea began to decrease and his appetite improved. A week later, his diarrhea was completely gone and he had a normal appetite. He was able to return to work and is receiving antiviral drug therapy. There appeared to be an upward trend of CD3, 4, 8 levels and the HIV count was undetectable.

[0152] At the time of the second clinical investigation, he had no symptoms of AIDS and his immune system appeared to be functioning normally. During TPA treatment according to the protocol of Example IV, he suffered no adverse effects. After treatment, his CD3, CD4 and CD8 levels increased somewhat while his white blood cell count remained unchanged. His HIV count continues to be undetectable. He is able to do strenuous work.

[0153] Patient #10 and #8a: W.F.W., Female, 47, participated in the first two studies, tested positive for HIV and had clear symptoms of the disease in 2003. Her symptoms at the initiation of the first study included low body temperature, diarrhea, low platelet count, coughing blood, bloody bowel movements, dizziness, headache, poor appetite, weight loss, fatigue with mild skin thrush and deep depression. She was hospitalized on one occasion for two months because of bloody bowel movements. She was very depressed and unable to work. She did not respond positively to the AZT, DDI and NVP treatment and her symptoms were not under control.

[0154] During her first treatment with TPA according to the protocol of Example III, she experienced an increase in body temperature to 38.5° C. on one occasion that lasted 4 hours. After TPA treatment, her dizziness, headache and diarrhea gradually lessened. Eventually, her appetite led to a weight gain and an improvement in her energy level. Her platelet count rose from 30,000 to 110,000 per microliter and the skin thrush and diarrhea were eliminated. She was able to work again and was treated with antiviral drugs. She had fever and diarrhea occasionally that she was able to control with drugs.

[0155] Six months later she suffered from mild headaches and dizziness and underwent a second treatment with TPA. During her second treatment with TPA, she experienced an increase in body temperature to 37.5 to 38° C. on five occasions that lasted 2 to 4 hours. Twenty hours after the 13^{th} injection of TPA, her temperature reached 40.5 degrees centigrade and lasted for several hours. It was concluded that the increase in temperature was not related to TPA therapy.

[0156] After her second treatment with TPA, her symptoms disappeared, her appetite improved and she gained weight, which enabled her to regain her energy, return to work and lead a normal life. She was free of symptoms for one year and has had few colds in the first six months after the second TPA treatment. There appears to be an upward trend for the CD 3, 4, and 8 levels and the HIV counts.

[0157] At the time of the second clinical trial according to the protocol of Example IV, this patient continued to display no symptoms of AIDS and her immune system appeared to be functioning normally. She suffered no adverse effects during treatment. After treatment, her CD3, CD4 and CD8 counts increased somewhat as did her WBC. Her HIV count increased somewhat. Since the studies, she has been healthy and engaged in laborious work.

[0158] Patient #11 and 9a: C.T.L., female, 40, participated in the first two studies, was diagnosed with AIDS and had clear symptoms of this disease in 2003. At the initiation of the first study she had persistent diarrhea, low body temperature, oral lesions, severe skin thrush, itching, purple blotches on her face and lips, dizziness, headache, poor appetite, and fatigue and depression. She responded poorly to AZT, 3TC and NVP treatment. Her symptoms were not under control and she was unable to work. Her initial CD4 count was 40.

[0159] During her first treatment with TPA, she experienced an increase in body temperature to 38 to 39° C. on four occasions that lasted 2 to 4 hours. She had shortness of breath on two occasions that lasted 20 to 30 minutes each.

[0160] After the sixth dose of TPA, her skin thrush began to disappear and upon completion of TPA treatment, the dizziness, headache, fever and skin thrush were improving and gradually faded away. Her appetite, physical condition and depression improved sufficiently for her to return to work.

[0161] This patient had a second treatment with TPA 18 months later due to the return of symptoms including mild skin thrush, diarrhea and dizziness. During this second treatment, she experienced an increase in body temperature to

37.5 to 38° C. three times that lasted 2 to 4 hours. There were no other adverse reactions. After treatment with TPA, her symptoms disappeared completely and her physical condition improved sufficiently to allow her to return to work. She has been without symptoms for one year and she has rarely had a cold. There appears to be an upward trend in CD 3, 4, and 8 levels, but her HIV counts did not change.

[0162] At the time of the second clinical study according to the protocol of Example IV, this patient exhibited symptoms of AIDS including headache, dizziness, poor appetite and a weak immune function. She suffered no adverse effects during treatment. After treatment, her CD3 and CD8 levels increased while her CD4 count was unchanged. Her HIV count increased slightly but no other changes were observed. Her mental and physical condition has improved considerably and she is doing strenuous physical work.

[0163] Patient #12 and #10a: C.C.L., female, 39, participated in the first two studies, diagnosed with AIDS and had clear symptoms of this disease in 2003. At the initiation of the first study she had persistent low body temperature, skin thrush, dizziness, headache, poor appetite, oral lesions, fatigue and deep depression. She was treated with AZT, 3TC and NVP but had poor results and she was unable to work. Her initial CD4 count was 84.

[0164] This patient was treated with TPA twice during the period March 2005 to March 2006. During the first treatment with TPA, she experienced an increase in body temperature to 38 to 38.5° C. on eight occasions that lasted 2 to 4 hours. She experienced shortness of breath on one occasion for 15 minutes and suffered a skin irritation due to a leaking needle.

[0165] After the seventh injection, her oral lesions disappeared. Upon completion of all the injections, all symptoms disappeared and her physical condition improved sufficiently for her to return to work.

[0166] Six months later, this patient was re-retreated with TPA due to the return of light diarrhea and dizziness. She experienced an increase in body temperature to 37.5 to 38° C. centigrade on six occasions associated with TPA administration that lasted 2 to 6 hours. Starting with the eighth injection, the dose was increased from approximately 150 μg to 250 μg TPA. No adverse effects occurred. Upon completion of TPA therapy, all her symptoms disappeared. Her physical condition was restored to normal and she returned to work and has had a normal life. She has been symptom free for one year and has rarely had a cold. There were no changes in CD 3, 4, or 8 levels, but her HIV count increased.

[0167] At the time of the second clinical study, this patient had no symptoms of AIDS though she did have a weakened immune system. She was treated according to the protocol of Example IV and suffered no adverse effects. After treatment, there were slight increases in her CD3, CD4 and CD8, and modest increases in WBC, RBC and HGB while platelets appeared to decrease. The HIV count was reduced somewhat. She has been healthy and engaged in strenuous physical work since her treatments.

[0168] Patient #13: L.F.L., female, 53, diagnosed with AIDS in 2004, participated in only the third clinical study. She presented with mild symptoms of poor appetite and weight loss. Long term antiviral drugs were effective and caused her virus count to decrease below detectable levels and CD3, CD4 and CD8 counts to increase to a high level. She had no symptoms prior to TPA treatment and had no side effects from its administration. She remains symptom free, feels normal, and is able to conduct normal activities. She restarted antiviral drug therapy after completion fo the 60 day observation period.

[0169] Patent #14: K.S.M., female, 45, diagnosed with AIDS in 2004, participated in only the third clinical study. Her symptoms were mild and consisted of poor appetite and frequent colds. She had been treated with antiviral drugs, but stopped due to severe liver toxicity. She had no symptoms prior TPA treatment and the only TPA side effect was irritation due to a leaking needle on day 43 that was easily treated. No AIDS symptoms occurred during the entire treatment and observation period. She feels normal and is able to conduct her usual activities. After completion of the 60 day observation period she was lost to the study and did not renew antiviral therapy.

Example VII

Treatment of Relapsed/Refractory Malignancies with TPA

[0170] Patients with histologically documented relapsed/refractory hematologic malignancy/bone marrow disorders are treated with a combination of TPA (Xichuan Pharmaceuticals, Nan Yang, Henan, China), dexamethasone and choline magnesium trisalicylate. Comparable methods as set forth below for demonstrating the therapeutic use of TPA in the treatment of Acute Myelogenous Leukemia (AML) will be applied to demonstrate the use of TPA for treating other neoplastic conditions and malignancies. Other neoplastic conditions and malignant disorders amenable to treatment using the methods and compositions of the invention include various forms of cancer, including blood and bone malignancies and solid tumors of various types. In addition to the specific protocols herein, successful treatment and/or remission will be determined for different targeted neoplastic and malignant conditions using any of a wide variety of well known cancer detection and assessment methods—for example by determining size reduction of solid tumors, histopathological studies to evaluate tumor growth, stage, metastatic potential, presence/expression levels of histological cancer markers, etc.

[0171] AML is an aggressive disease that generally warrants urgent and intensive therapy. The average patient age at AML diagnosis is 64-68 years old, and patients over the age of 60 treated with standard chemotherapy are cured of their disease<20% of the time. Patients who develop AML after an antecedent hematologic disorder or prior leukemogenic chemotherapy/radiation therapy have similarly poor outcomes, as do patients whose disease is associated with specific adverse cytogenetic and clinical features. Hence, most patients diagnosed with AML have patient and/or disease-related features that are associated with a very poor prognosis. For patients with relapsed disease, no standard non-transplant therapy has demonstrated the capacity for cure. For these patients, AML is often a fatal disease. New approaches to the therapy of AML are needed.

[0172] Employing the methods and compositions of the instant invention, TPA, is developed as a therapeutic agent for treating patients with AML, based on TPA's novel role in modulating intracellular signaling pathways, it's capacity to induce differentiation and/or apoptosis in cell lines, and clinical data indicating the effectiveness of TPA in treating neoplastic and malignant disorders, including myeloid malignancies.

[0173] Thus far clinical evaluation of TPA has demonstrated that TPA exerts direct therapeutic cytotoxic effects in at least a subset of AML cases, as measured by cell viability and apoptosis assays. In all primary cultures analyzed by Western analysis, TPA strongly induced ERK phosphorylation by 1 hour in culture. TPA's cytotoxic effect on primary AML cells is associated with the subsequent loss of the phospho-ERK pro-survival signal after 24 hour ex vivo exposure. This observation is in good agreement with other studies that reported decreased primary AML survival after pharmacological interruption of ERK signaling by MEK inhibitors, such as PD98059, U0126 and PD 184352. In our studies, loss of ERK signaling was associated with induction of ERK phosphatases.

[0174] In addition to protein kinase C and ERK activation, TPA is a known inducer of NF-κB, a pro-survival transcription factor often constitutively active in AML blasts and leukemic stem cells. Recent work from our laboratory has demonstrated that AML cell NF-κB can be inhibited in vivo with 48 h of treatment with dexamethasone+choline magnesium trisalicylate (CMT). In addition, we have shown that dexamethasone can induce MKP-1 ERK phosphatase expression and enhance TPA cytotoxicity on primary AML samples. In this context, we have chosen in exemplary embodiments below to use dexamethasone and CMT as adjunctive medications to be used 24 h pre- and 24 h post treatment with TPA. These medications are well-tolerated and anticipated to reduce inflammatory adverse effects of treatment and enhance TPA cytotoxicity by increasing ERK phosphatase expression and inhibiting NF-κB. In addition dexamethasone and CMT will be used as adjunctive medications because they are anti-inflammatory, may ameliorate adverse effects, and may enhance anti-leukemic activity by inhibition of the anti-apoptotic effects of constitutive NF-κB expression and induction of phosphatases that decrease signaling pathway activity.

[0175] An initial TPA Phase 1 study enrolled 35 patients [23 with relapsed/refractory AML, 2 with other myeloid malignancies (CML-blast crisis, myelodysplasia with excess blasts), 3 with Hodgkin's Disease, 3 with non-Hodgkin's lymphoma and 4 with solid tumors]. The majority of patients had relapsed/refractory AML. Our clinical results include one AML patient with stable disease for >5 months, who received 8 TPA infusions. In a second AML patient, a pronounced (5-fold) decline in the number of circulating blasts was seen following TPA administration. This decline in leukemic blasts persisted for 4 weeks, and the patient eventually died from a fungal infection. Finally, a patient with relapsed and refractory Hodgkin's disease despite high dose chemotherapy with autologous stem cell rescue had a partial remission of a chest wall mass after TPA administration. TPA dose escalation has been completed, in the last cohort 2 out of 3 patients treated at a dose of 0.188 mg/m2 d1-5, 8-12 experienced grade III non-hematologic dose limiting toxicities (DLT), establishing the maximum tolerated TPA dose as a single agent at 0.125 mg/m2/d on d1-5 and 8-12.

[0176] In the case of AML and other hematologic malignancies, patients are given an initial dose of TPA of 1 mg/week×3 weeks (days 1, 8, 15) administered with continuous/intermittent pulse oximetry for 6 hours. Twenty four hours prior to initiation of TPA therapy, patients are given 10 mg of dexamethasone every six hours and 1500 mg of choline magnesium trisalicylate (CMT) every eight hours continuing until 24 hours after administration of TPA. After administration of the initial dose of TPA, patients have a two week rest period after which they may be reevaluated. Those patients that have a disease response or stabilization from the initial dose of TPA are treated for up to six cycles of twenty-eight days according to the protocol below.

[0177] Following the two week rest period, patients are pre-medicated with Tylenol 650 mg and Benadryl 25-50 mg (depending on the patient's size and age) thirty minutes prior to administration of TPA. They are then given an intravenous infusion of TPA through a central venous catheter daily for 5 days a week for two consecutive weeks followed by a 2-week rest period. TPA is administered at a dose of 1 mg in 200 ml of normal saline over 1 hour. Twenty four hours prior to initiation of TPA therapy, patients are given 10 mg of dexamethasone every six hours and 1500 mg of choline magnesium trisalicylate continuing every eight hours until 24 hours after administration of the TPA.

[0178] Blood levels of TPA are measured prior to and after infusion using a bioassay that measures organic solvent extractable differentiation activity. 1 ml of blood is extracted twice with 5 ml of ethyl acetate, redissolving the extraction residue in 50 μL of ethanol and addition of an aliquot of HL60 cells. After 48 hours, adherent cells are measured.

[0179] Tests are also run on blood samples taken prior to and after infusion with TPA to determine levels of white blood cells, platelets, and neutrophils. The samples are additionally analyzed for the presence of myeloblasts and Auer rods. These and continuing experiments will further elucidate the therapeutic cytotoxic and other effects that TPA elicits against neoplastic cells in AML and other neoplastic and malignant conditions.

Example VIII

Measurement of the Modulation of ERK Activation

[0180] Phospho-ERK levels are measured in circulating malignant cells in patients with leukemia and in peripheral blood mononuclear cells in lymphoma/solid tumor patients. A blood sample is taken from patients treated according to the protocol of Example VII both prior to and after administration of TPA.

[0181] In leukemia patients with a WBC≥1000 per μL, flow cytometry is performed on a blood sample using cell surface antigen-specific and phospho-ERK specific antibodies directly conjugated to fluorophores (BD Biosciences, San Jose, Calif.). Samples are taken pre-administration of TPA and one our after infusion of TPA on days 1, 2, and 11 in the initial treatment according to the protocol of Example VII and days 1 and 11 in subsequent cycles. In leukemia patients with an absolute leukemic blast number≥2500 per μL and other non-leukemic patients, peripheral blood samples are taken on days 1, 8 and 15 of the initial cycle according to the protocol of Example VII prior to and 1 and 4 hours post infusion. Samples are also analyzed using Western blot analysis for phosphor-ERK, and total ERK1/2 levels to confirm the results obtained from the flow cytometry and correlated to clinical responses.

[0182] The foregoing analyses will further elucidate TPA's role in treatment of neoplastic and malignant conditions, including TPA's cytotoxic effect on malignant cells, exemplified by primary AML cells, and the associated reduction by TPA of the phosphor-ERK pro-survival signal.

Example IX

Measurement of NF-κB modulation

[0183] In prior studies we have shown that NF-κB activity can be modulated in patients following administration of TPA with dexamethasone. Additionally, dexamethasone has been shown to induce MKP-1 ERK phosphatase expression and enhance TPA cytotoxicity. The following studies are designed to further elucidate how NF-κB activity is therapeutically modulated in patients treated with TPA plus dexamethasone.

[0184] NF-κB binding is measured in patient peripheral blood samples at baseline and pre and post infusion from patients treated with TPA according to Example VII using ELISA-based assays (BD Bioscience, San Jose, USA). NF-κB levels are quantified using chemiluminescent intensity to detect binging in limiting amounts of cellular extract using a 96-well format. Additionally, electrophoretic mobility shift assays are performed to measure NF-κB binding in peripheral blood samples from leukemia patient with an absolute leukemic blast number≥2500 per μL and other non-leukemic patients with normal white blood cell counts.

[0185] The foregoing studies will further PA is an inducer of NF-κB, however these experiments demonstrate that AML cell NF-κB can be inhibited with treatment with dexamethasone and choline magnesium trisalicylate.

Example X

Determination of changes in Leukemic Gene Expression

[0186] TPA induces RNA levels of several dual specificity phosphatases capable of terminating pro-survival ERK pathway signaling. A blood sample taken pre and post infusion from patients with AML treated with TPA according to Example VII is used to study RNA expression of AML signaling components such as the MAPK-specific DUSPs using quantitative realtime RT-PCR and oligonucleotide microarray analysis.

[0187] Although the foregoing invention has been described in detail by way of example for purposes of clarity of understanding, it will be apparent to the artisan that certain changes and modifications may be practiced within the scope of the appended claims which are presented by way of illustration not limitation. In this context, various publications and other references have been cited with the foregoing disclosure for economy of description. Each of these references is incorporated herein by reference in its entirety for all purposes. It is noted, however, that the various publications discussed herein are incorporated solely for their disclosure prior to the filing date of the present application, and the inventors reserve the right to antedate such disclosure by virtue of prior invention.

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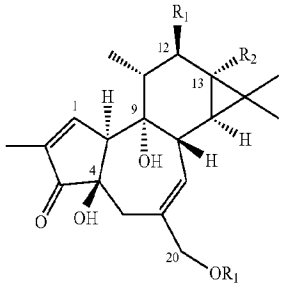
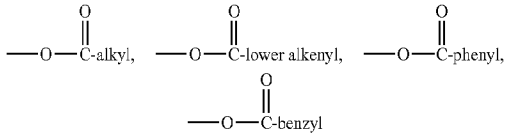
We claim:

1. A method for preventing or treating HIV infection or disease in a mammalian subject comprising administering an effective amount of a phorbol ester or derivative of Formula I, or a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, polymorph or prodrug thereof to said subject

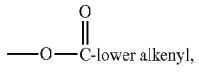
Formula 1



wherein R_1 and R_2 are selected from the group consisting of hydrogen,

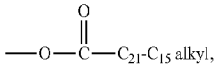


and substituted derivatives thereof, and R_3 is selected from hydrogen,

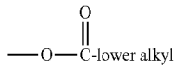


and substituted derivatives thereof.

2. The method of claim **1**, wherein R_1 or R_2 is



the remaining R_1 or R_2 is



and R_3 is hydrogen.

3. The method of claim **1**, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

4. The method of claim **1**, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

5. The method of claim **1**, further comprising administering at least one secondary anti-retroviral or other adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester or derivative compound of Formula I to treat or prevent HIV in said subject.

6. The method of claim **5**, wherein the at least one secondary anti-retroviral or other adjunctive therapeutic agent is administered to said subject in a coordinate administration protocol, simultaneously with, prior to, or after, administration of said phorbol ester to said subject.

7. The method of claim **5**, wherein the at least one secondary anti-retroviral or other adjunctive therapeutic agent is selected from the group consisting of: protease inhibitors, nucleoside reverse transcriptase, non-nucleoside reverse transcriptase inhibitors, combination drugs, entry and fusion inhibitors, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, paclitaxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, valganciclovir; integrase inhibitors, microbicides, and IL-2.

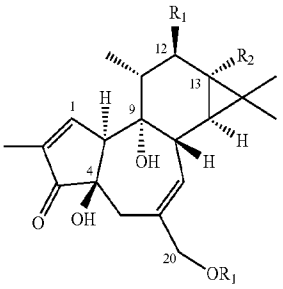
8. The method of claim **1**, wherein said effective amount comprises between about 10 and 1500 μg of said phorbol ester or derivative compound of Formula I every other day.

9. The method of claim **1**, wherein said effective amount comprises between about 150 to 500 μg of said phorbol ester or derivative compound of Formula I every other day.

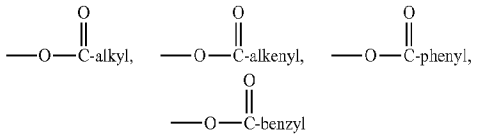
10. The method of claim **1**, wherein said effective amount of said phorbol ester compound or derivative compound of Formula I is administered once per day.

11. A method for preventing or treating one or more symptoms or conditions of HIV infection or AIDS in a mammalian subject comprising administering an effective amount of phorbol ester or derivative compound of Formula I, or a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, polymorph or prodrug thereof to said subject

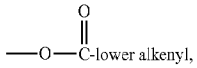
Formula I



wherein R_1 and R_2 are selected from the group consisting of hydrogen,

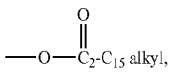


and substituted derivatives thereof, and R_3 is selected from hydrogen,

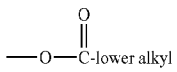


and substituted derivatives thereof.

12. The method of claim **11**, wherein R_1 or R_2 is



alkyl, the remaining R_1 or R_2 is



and R_3 is hydrogen.

13. The method of claim **11**, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

14. The method of claim **11**, wherein the phorbol ester is 12-O-tetradecanoylphobol-13-acetate.

15. The method of claim **11**, further comprising administering at least one secondary anti-retroviral or other adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester or derivative compound of Formula I to treat symptoms or conditions of AIDS in said subject.

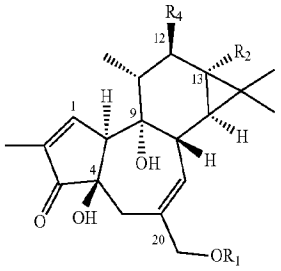
16. The method of claim **11**, wherein the at least one secondary anti-retroviral or other adjunctive therapeutic agent is administered to said subject in a coordinate administration protocol, simultaneously with, prior to, or after, administration of said phorbol ester to said subject.

17. The method of claim **11**, wherein the at least one secondary anti-retroviral or other adjunctive therapeutic agent is selected from the group consisting of: protease inhibitors, nucleoside reverse transcriptase, non-nucleoside reverse transcriptase inhibitors, combination drugs, entry and fusion inhibitors, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, paclitaxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, valganciclovir; integrase inhibitors, microbicides, and IL-2.

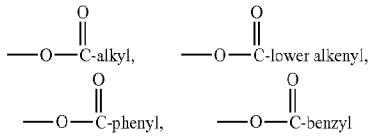
18. The method of claim **11**, wherein the one or more symptoms or conditions of AIDS are oral lesions, fatigue, skin thrush, fever, lack of appetite, diarrhea, apthous ulcers, malabsorbtion, thrombocytopenia, weight loss, anemia, and lymph node enlargement, *mycobacterium avium* complex, salmonellosis, syphilis, neuroshyphilis, turberculosis, bacillary angiomatosis, aspergillosis, candidiasis, coccidioidomycosis, listeriosis, pelvic inflammatory disease, Burkitt's lymphoma, cryptococcal meningitis, histoplasmosis, Kaposi's sarcoma, lymphoma, systemic non-Hodgkin's lymphoma, primary CNS lymphoma, cryptosporidiosis, isosporiasis, microsporidiosis, *pneumocystis carinii* pneumonia, toxoplasmosis, cytomegalovirus, hepatitis, herpes simplex, herpes zoster, human papiloma virus, molluscum contagiosum, oral hairy leukoplakia, and progressive multifocal leukoencephalopathy.

19. A method for controlling HIV infection in a mammalian subject to reduce or prevent AIDS comprising administering to said subject an effective amount of a phorbol ester or derivative compound of Formula I, or a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, polymorph or prodrug thereof, to said subject

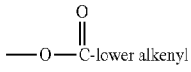
Formula I



wherein R_1 and R_2 are selected from the group consisting of hydrogen,

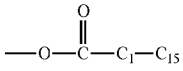


and substituted derivatives thereof and R_3 is selected from hydrogen,

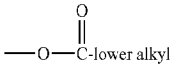


and substituted derivatives thereof.

20. The method of claim **19**, wherein R_1 or R_2 is



alkyl, the remaining R_1 or R_2 is



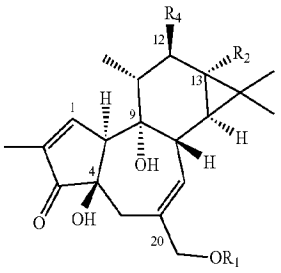
and R_3 is hydrogen.

21. The method of claim **19**, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

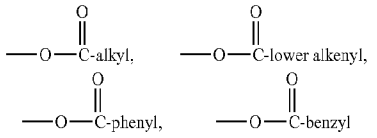
22. The method of claim **19**, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

23. A composition for preventing or alleviating HIV in a mammalian subject comprising an effective amount of a phorbol ester or derivative compound of Formula I, or a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, polymorph or prodrug thereof

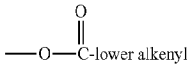
Formula I



wherein R_1 and R_2 are selected from the group consisting of hydrogen,

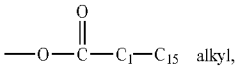


and substituted derivatives thereof, and R_3 is selected from hydrogen,

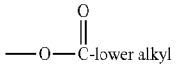


and substituted derivatives thereof.

24. The composition of claim **23**, wherein R_1 or R_2 is



the remaining R_1 or R_2 is



and R_3 is hydrogen.

25. The composition of claim **23**, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate,

12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

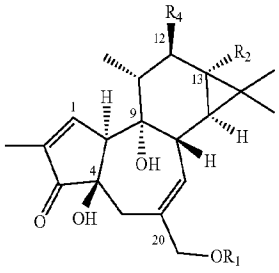
26. The composition of claim **23**, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

27. The composition of claim **23**, further comprising at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation with said phorbol ester or derivative compound of Formula I.

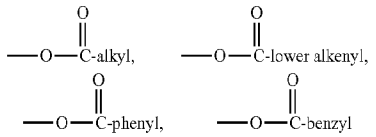
28. The composition of claim **23**, wherein the at least one secondary or adjunctive therapeutic agent is selected from the group consisting of protease inhibitors, nucleoside reverse transcriptase, non-nucleoside reverse transcriptase inhibitors, combination drugs, entry and fusion inhibitors, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, paclitaxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, valganciclovir; integrase inhibitors, microbicides, and IL-2.

29. A method for activating latent reservoirs of HIV comprising administering an effective amount of a phorbol ester or derivative compound of Formula I, or a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, polymorph or prodrug thereof to said subject

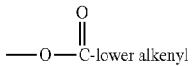
Formula I



wherein R_1 and R_2 are selected from the group consisting of hydrogen,

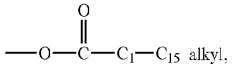


and substituted derivatives thereof, and R_3 is selected from hydrogen,

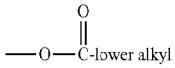


and substituted derivatives thereof.

30. The method of claim **29**, wherein R_1 or R_2 is



the remaining R_1 or R_2 is



and R_3 is hydrogen.

31. The method of claim **29**, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

32. The method of claim **29**, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

33. The method of claim **29**, further comprising administering a secondary anti-retroviral or other adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester or related or derivative compound of Formula I.

34. The method of claim **33**, wherein the secondary anti-retroviral or adjunctive therapeutic agent is administered to said subject in a coordinate administration protocol, simultaneously with, prior to, or after, administration of said phorbol ester to said subject.

35. The method of claim **33**, wherein the secondary anti-retroviral or adjunctive therapeutic agent is selected from the group consisting of: protease inhibitors, nucleoside reverse transcriptase, non-nucleoside reverse transcriptase inhibitors, combination drugs, entry and fusion inhibitors, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, paclitaxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, valganciclovir; integrase inhibitors, microbicides, and IL-2.

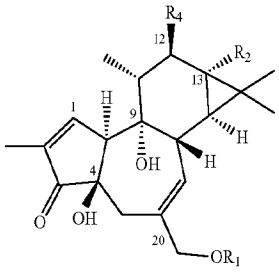
36. The method of claim **29**, wherein said effective amount comprises between about 10 and 1500 μg of said phorbol ester or derivative compound of Formula I every other day.

37. The method of claim **29**, wherein said effective amount comprises between about 150 to 500 μg of said phorbol ester or derivative compound of Formula I every other day.

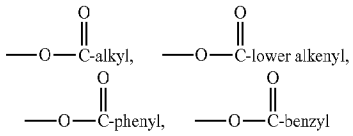
38. The method of claim **29**, wherein said effective amount of said phorbol ester compound or derivative compound of Formula I is administered once per day.

39. A method of increasing the expression of Th1 cytokines comprising administering an effective amount of a phorbol ester or derivative of Formula I, or a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, polymorph or prodrug thereof to said subject

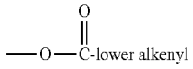
Formula I



wherein R_1 and R_2 are selected from the group consisting of hydrogen,

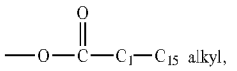


and substituted derivatives thereof, and R_3 is selected from hydrogen,

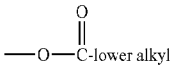


and substituted derivatives thereof.

40. The method of claim **39**, wherein R_1 or R_2 is



the remaining R_1 or R_2 is



and R_3 is hydrogen.

41. The method of claim **39**, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

42. The method of claim **39**, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

43. The method of claim **39**, further comprising administering a secondary or other adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester or derivative compound of Formula I to upregulate Th1 cytokines.

44. The method of claim **43**, wherein the secondary or adjunctive therapeutic agent is administered to said subject in a coordinate administration protocol, simultaneously with, prior to, or after, administration of said phorbol ester to said subject.

45. The method of claim **43**, wherein the secondary or adjunctive therapeutic agent is selected from the group consisting of: protease inhibitors, nucleoside reverse transcriptase, non-nucleoside reverse transcriptase inhibitors, combination drugs, entry and fusion inhibitors, acyclovir, adefovir dipivoxil, aldesleukin, amphotericin b, azithromycin, calcium hydroxylapatite, clarithromycin, doxorubicin, dronabinol, entecavir, epoetin alfa, etoposide, fluconazole, ganciclovir, immunoglobulins, interferon alfa-2, isoniazid, itraconazole, megestrol, paclitaxel, peginterferon alfa-2, pentamidine, poly-1-lactic acid, ribavirin, rifabutin, rifampin, somatropin, testosterone, trimetrexate, valganciclovir; integrase inhibitors, microbicides, and IL-2.

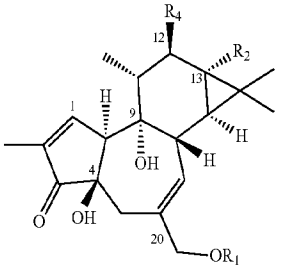
46. The method of claim **39**, wherein said effective amount comprises between about 10 and 1500 μg of said phorbol ester or derivative compound of Formula I every other day.

47. The method of claim **39**, wherein said effective amount comprises between about 150 to 500 μg of said phorbol ester or derivative compound of Formula I every other day.

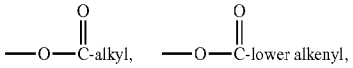
48. The method of claim **40**, wherein said effective amount of said phorbol ester compound or derivative compound of Formula I is administered once per day.

49. A method of treating or preventing neoplasms in a mammalian subject comprising administering an effective amount of a phorbol ester or derivative of Formula I, or a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, polymorph or prodrug thereof to said subject

Formula I



wherein R_1 and R_2 are selected from the group consisting of hydrogen,



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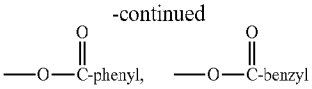
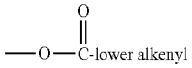
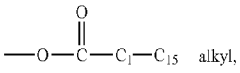


and substituted derivatives thereof, R_3 is selected from hydrogen,

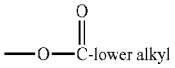


and substituted derivatives thereof; and at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester or derivative compound of Formula I to treat or prevent neoplasms in said subject.

50. The method of claim **49**, wherein R_1 or R_2 is



the remaining R_1 or R_2 is



and R_3 is hydrogen.

51. The method of claim **49**, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

52. The method of claim **49**, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

53. The method of claim **49**, wherein the at least one secondary or adjunctive therapeutic agent is administered to said subject in a coordinate administration protocol, simultaneously with, prior to, or after, administration of said phorbol ester to said subject.

54. The method of claim **49**, wherein the at least one secondary or adjunctive therapeutic agent is selected from the group consisting of: doxorubicin, vitamin D3, cytarabine, cytosine arabinoside, daunorubicin, cyclophosphamide, gemtuzumab ozogamicin, idarubicin, mercaptopurine, mitoxantrone, thioguanine, aldesleukin, asparaginase, carboplatin, etoposide phosphate, fludarabine, methotrexate, etoposide, dexamethasone, and choline magnesium trisalicylate.

55. The method of claim **49**, wherein two secondary or adjunctive therapeutic agents are administered to said subject.

56. The method of claim **55**, wherein the two secondary or adjunctive therapeutic agents are dexamethasone and choline magnesium trisalicylate.

57. The method of claim **49**, wherein said effective amount comprises between about 10 and 1500 μg of said phorbol ester or derivative compound of Formula I every day.

58. The method of claim **49**, wherein said effective amount comprises between about 150 to 500 μg of said phorbol ester or derivative compound of Formula I every day.

59. The method of claim **49** wherein the neoplasm is caused by a hematological malignancy/bone marrow disorder.

60. The method of claim **59**, wherein the hematological malignancy/bone marrow disorder is leukemia.

61. The method of claim **60**, wherein the leukemia is acute myeloid leukemia.

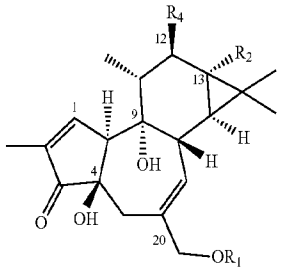
62. The method of claim **49**, wherein the neoplasm is a solid tumor.

63. The method of claim **49**, wherein the neoplasm is a relapsing neoplasm.

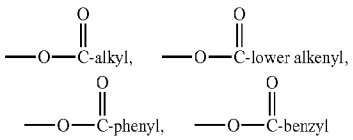
64. The method of claim **49**, wherein the neoplasm is refractory.

65. A method for preventing or treating one or more symptoms or conditions of neoplastic disease in a mammalian subject comprising administering an effective amount of phorbol ester or derivative compound of Formula I, or a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, polymorph or prodrug thereof to said subject

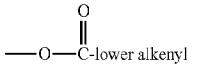
Formula I



wherein R_1 and R_2 are selected from the group consisting of hydrogen,



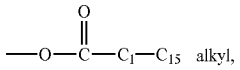
and substituted derivatives thereof, R_3 is hydrogen,



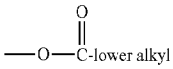
and substituted derivatives thereof; and at least one secondary or adjunctive therapeutic agent that is effective in a combina-

torial formulation or coordinate treatment regimen with said phorbol ester or derivative compound of Formula I to treat or prevent symptoms of neoplastic disease in said subject.

66. The method of claim **65**, wherein R_1 or R_2 is



the remaining R_1 or R_2 is



and R_3 is hydrogen.

67. The method of claim **65**, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

68. The method of claim **65**, wherein the phorbol ester is 12-O-tetradecanoylphobol-13-acetate.

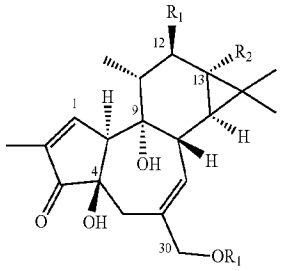
69. The method of claim **68**, wherein the at least one secondary or adjunctive therapeutic agent is administered to said subject in a coordinate administration protocol, simultaneously with, prior to, or after, administration of said phorbol ester to said subject.

70. The method of claim **68**, wherein the at least one secondary or adjunctive therapeutic agent is selected from the group consisting of: doxorubicin, vitamin D3, cytarabine, cytosine arabinoside, daunorubicin, cyclophosphamide, gemtuzumab ozogamicin, idarubicin, mercaptopurine, mitoxantrone, thioguanine, aldesleukin, asparaginase, carboplatin, etoposide phosphate, fludarabine, methotrexate, etoposide, dexamethasone, and choline magnesium trisalicylate.

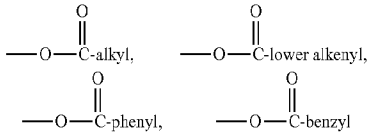
71. The method of claim **68**, wherein the one or more symptoms or conditions of neoplastic disease are anemia, chronic fatigue, excessive or easy bleeding, easy bruising, shortness of breath, petechiae, recurrent fever, swollen gums, slow healing of cuts, bone and joint discomfort, recurrent infections, weight loss, itching, night sweats, lymph node swelling, fever, abdominal pain and discomfort, disturbances in vision, coughing, loss of appetite, pain in the chest, difficulty swallowing, swelling, a need to urinate frequently, difficulty starting urination, difficulty holding back urine, weak or interrupted flow of urine, painful or burning urination, difficulty in having an erection, painful ejaculation, blood in urine or semen, frequent pain or stiffness, or weakness.

72. A method for inducing remission in a mammalian subject suffering from neoplastic disease comprising administering to said subject an effective amount of a phorbol ester or derivative compound of Formula I, or a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, polymorph or prodrug thereof, to said subject

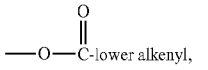
Formula I



wherein R_1 and R_2 are selected from the group consisting of hydrogen,

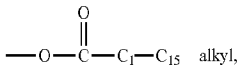


and substituted derivatives thereof; R_3 is hydrogen,

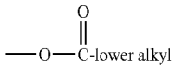


and substituted derivatives thereof; and at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester of derivative compound of Formula I to induce remission in said subject.

73. The method of claim **72**, wherein R_1 or R_2 is



the remaining R_1 or R_2 is



and R_3 is hydrogen and at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester or derivative compound of Formula I to induce remission in said subject.

74. The method of claim **72**, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol

13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

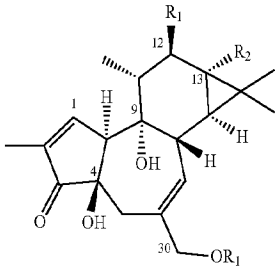
75. The method of claim **72**, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

76. The method of claim **72**, wherein the at least one secondary or adjunctive therapeutic agent is administered to said subject in a coordinate administration protocol, simultaneously with, prior to, or after, administration of said phorbol ester to said subject.

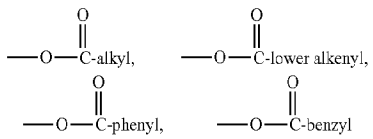
77. The method of claim **72**, wherein the at least one secondary or adjunctive therapeutic agent is selected from the group consisting of: doxorubicin, vitamin D3, cytarabine, cytosine arabinoside, daunorubicin, cyclophosphamide, gemtuzumab ozogamicin, idarubicin, mercaptopurine, mitoxantrone, thioguanine, aldesleukin, asparaginase, carboplatin, etoposide phosphate, fludarabine, methotrexate, etoposide, dexamethasone, and choline magnesium trisalicylate.

78. A method for inducing apoptosis in a neoplasm in a mammalian subject suffering from neoplastic disease comprising administering to said subject an effective amount of a phorbol ester or derivative compound of Formula I, or a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, polymorph or prodrug thereof, to said subject

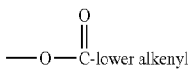
Formula I



wherein R_1 and R_2 are selected from the group consisting of hydrogen,

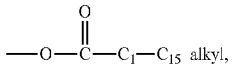


and substituted derivatives thereof, R_3 is hydrogen,

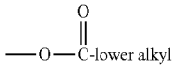


and substituted derivatives thereof; and at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester of derivative compound of Formula I to induce apoptosis in a neoplasm in said subject.

79. The method of claim **78**, wherein R_1 or R_2 is



the remaining R_1 or R_2 is



and R_3 is hydrogen and at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation or coordinate treatment regimen with said phorbol ester or derivative compound of Formula I to treat or prevent malignancy in said subject.

80. The method of claim **78**, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

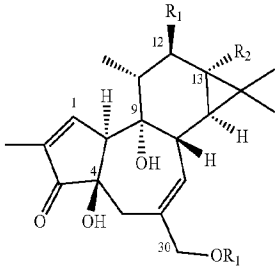
81. The method of claim **78**, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

82. The method of claim **78**, wherein the at least one secondary or adjunctive therapeutic agent is administered to said subject in a coordinate administration protocol, simultaneously with, prior to, or after, administration of said phorbol ester to said subject.

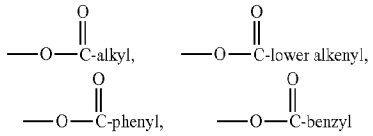
83. The method of claim **82**, wherein the at least one secondary or adjunctive therapeutic agent is selected from the group consisting of: doxorubicin, vitamin D3, cytarabine, cytosine arabinoside, daunorubicin, cyclophosphamide, gemtuzumab ozogamicin, idarubicin, mercaptopurine, mitoxantrone, thioguanine, aldesleukin, asparaginase, carboplatin, etoposide phosphate, fludarabine, methotrexate, etoposide, dexamethasone, and choline magnesium tri salicylate.

84. A composition for preventing or treating neoplastic disease in a mammalian subject comprising an effective amount of a phorbol ester or derivative compound of Formula I, or a pharmaceutically-acceptable salt, isomer, enantiomer, solvate, hydrate, polymorph or prodrug thereof

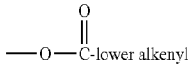
Formula I



wherein R_1 and R_2 are selected from the group consisting of hydrogen,

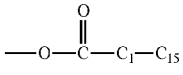


and substituted derivatives thereof, R_3 is hydrogen,

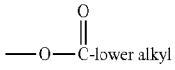


and substituted derivatives thereof; and at least one secondary or adjunctive therapeutic agent that is effective in a combinatorial formulation with said phorbol ester or derivative compound of Formula I to treat or prevent a neoplasm in said subject.

85. The composition of claim **84**, wherein R_1 or R_2 is



alkyl, the remaining R_1 or R_2 is



and R_3 is hydrogen.

86. The composition of claim **84**, wherein the phorbol ester is phorbol 13-butyrate, phorbol 12-decanoate, phorbol 13-decanoate, phorbol 12,13-diacetate, phorbol 13,20-diacetate, phorbol 12,13-dibenzoate, phorbol 12,13-dibutyrate, phorbol 12,13-didecanoate, phorbol 12,13-dihexanoate, phorbol 12,13-dipropionate, phorbol 12-myristate, phorbol 13-myristate, phorbol 12,13,20-triacetate, 12-deoxyphorbol 13-angelate, 12-deoxyphorbol 13-angelate 20-acetate, 12-deoxyphorbol 13-isobutyrate, 12-deoxyphorbol 13-isobutyrate-20-acetate, 12-deoxyphorbol 13-phenylacetate, 12-deoxyphorbol 13-phenylacetate 20-acetate, 12-deoxyphorbol 13-tetradecanoate, phorbol 12-tigliate 13-decanoate, 12-deoxyphorbol 13-acetate, phorbol 12-acetate, or phorbol 13-acetate.

87. The composition of claim **84**, wherein the phorbol ester is 12-O-tetradecanoylphorbol-13-acetate.

88. The composition of claim **84**, wherein the at least one secondary or adjunctive therapeutic agent is selected from the group consisting of: doxorubicin, vitamin D3, cytarabine, cytosine arabinoside, daunorubicin, cyclophosphamide, gemtuzumab ozogamicin, idarubicin, mercaptopurine, mitoxantrone, thioguanine, aldesleukin, asparaginase, carboplatin, etoposide phosphate, fludarabine, methotrexate, etoposide, dexamethasone, and choline magnesium trisalicylate.

89. The composition of claim **84**, wherein the composition contains at least two secondary or adjunctive therapeutic agents.

90. The composition of claim **89**, wherein the at least two secondary or adjunctive therapeutic agents are dexamethasone and choline magnesium trisalicylate.

* * * * *