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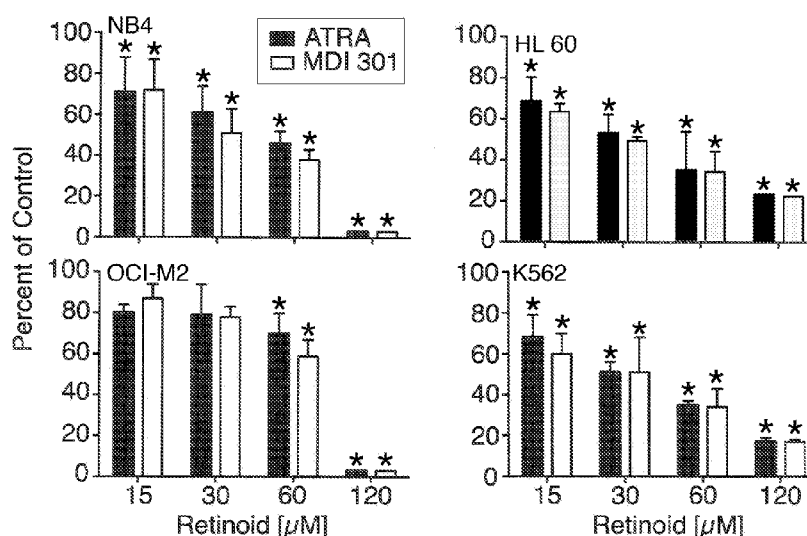
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(54) Title: INHIBITORS OF MYELOID LEUKEMIA CELL GROWTH AND RELATED METHODS

FIG. 1A



(57) Abstract: Methods of treatment using inhibitors of myeloid leukemia cell growth are provided. Such methods include, but are not limited to, methods of treating myeloid leukemia (e.g., promyelocytic leukemia, acute myeloid leukemia). Such inhibitors of myeloid leukemia cell growth include, but are not limited to, pharmaceutical compositions including a cis- derivative(s) of retinoic acid (e.g., pinacolyl 9-cw-retinoate, (2E, 4E, 6Z, 8E)-3,3-Dimethyl-2- xo-1-butyl 3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nonatetraenoate (MDI 301)). Related kits and compositions are further provided.

INHIBITORS OF MYELOID LEUKEMIA CELL GROWTH AND RELATED METHODS

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit U.S. Provisional Patent Application No. 62/115,397 filed February 12, 2015, the contents of which is incorporated by reference in its entirety.

FIELD OF THE INVENTION

10 Methods of treatment using inhibitors of myeloid leukemia cell growth are provided. Such methods include, but are not limited to, methods of treating myeloid leukemia (e.g., promyelocytic leukemia, acute myeloid leukemia). Such inhibitors of myeloid leukemia cell growth include, but are not limited to, pharmaceutical compositions including a *cis*-
15 derivative(s) of retinoic acid (e.g., pinacolyl 9-*cis*-retinoate, (2E, 4E, 6Z, 8E)-3,3-Dimethyl-2-xo-1-butyl 3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nonatetraenoate (MDI 301)). Related kits and compositions are further provided.

BACKGROUND OF THE INVENTION

20 Myeloid leukemia is a type of leukemia affecting myeloid tissue. Examples of myeloid leukemia include, but are not limited to, promyelocytic leukemia and acute myeloid leukemia.

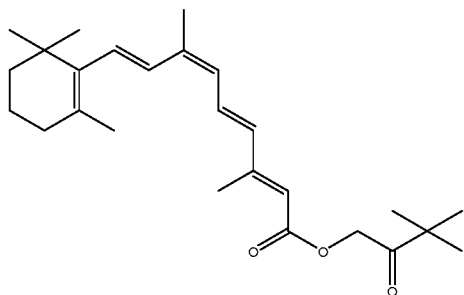
 Acute myeloid leukemia (AML) is malignant disease of the bone marrow in which hematopoietic precursors are arrested in an early stage of development. Most AML subtypes are distinguished from other related blood disorders by the presence of more than 20% blasts
25 in the bone marrow. The underlying pathophysiology in AML consists of a maturational arrest of bone marrow cells in the earliest stages of development. Several factors have been implicated in the causation of AML, including antecedent hematologic disorders, familial syndromes, environmental exposures, and drug exposures. However, most patients who present with de novo AML have no identifiable risk factor.

30 Promyelocytic leukemia (PML) (also referred to as acute promyelocytic leukemia (APL)) is a unique subtype of AML. It has distinct cytogenetics, clinical features, and biologic characteristics. In PML, there is an abnormal accumulation of immature granulocytes called promyelocytes. The disease is characterized by a chromosomal translocation involving the retinoic acid receptor alpha (RAR α or RARA) gene.

Improved methods for treating and understanding myeloid leukemia are needed.

SUMMARY OF THE INVENTION

Pinacolyl 9-*cis*-retinoate, (2E, 4E, 6Z, 8E)-3,3-Dimethyl-2-oxo-1-butyl 3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nonatetraenoate (MDI 301):



, an RAR α – agonist, is a 9-*cis* retinoic acid derivative in which the terminal carboxylic acid group has been replaced by a picolinate ester (see, e.g., U.S. Patent No. 5,837,728).

In experiments conducted during the course of developing embodiments for the present invention, MDI 301 was shown to suppress growth of several human myeloid leukemia cell lines (e.g., HL60, NB4, OCI-M2 and K562) *in vitro* and slow the growth of xenografted tumor growth (HL60) in athymic nude mice. *In vitro*, MDI 301 was shown to be comparable to all-*trans* retinoic acid (ATRA) while *in vivo*, MDI 301 was slightly more efficacious than ATRA. Unlike what was seen with ATRA treatment, MDI 301 did not induce a cytokine response in the treated animals and the severe inflammatory changes and systemic toxicity typically seen with ATRA did not occur.

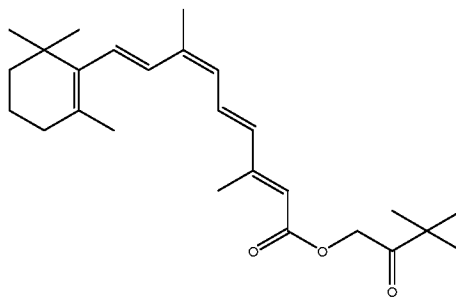
Accordingly, the present invention provides methods of treatment using inhibitors of myeloid leukemia cell growth. Such methods include, but are not limited to, methods of treating myeloid leukemia (e.g., promyelocytic leukemia, acute myeloid leukemia). Such inhibitors of myeloid leukemia cell growth include, but are not limited to, pharmaceutical compositions including a *cis*-derivative(s) of retinoic acid (e.g., pinacolyl 9-*cis*-retinoate, (2E, 4E, 6Z, 8E)-3,3-Dimethyl-2-oxo-1-butyl 3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nonatetraenoate (MDI 301)). Related kits and compositions are further provided.

In certain embodiments, the present invention provides methods for treating, ameliorating, or preventing recurrence of a condition involving myeloid leukemia cell growth in a patient (e.g., a human patient) comprising administering to the patient a therapeutically effective amount of a *cis*-derivative of retinoic acid, including salts, esters and prodrugs thereof, and a pharmaceutically acceptable carrier.

Such methods are not limited to a particular *cis*-derivative of retinoic acid. In some embodiments, the *cis*-derivative of retinoic acid upon administration to the patient does not induce a cytokine response, an inflammatory response, and/or systemic toxicity in the patient. In some embodiments, the *cis*-derivative of retinoic acid upon administration to the patient

5 does not induce retinoic acid syndrome in the patient.

In certain embodiments, the *cis*-derivative of retinoic acid is pinacolyl 9-*cis*-retinoate, (2E, 4E, 6Z, 8E)-3,3-Dimethyl-2-oxo-1-butyl 3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-

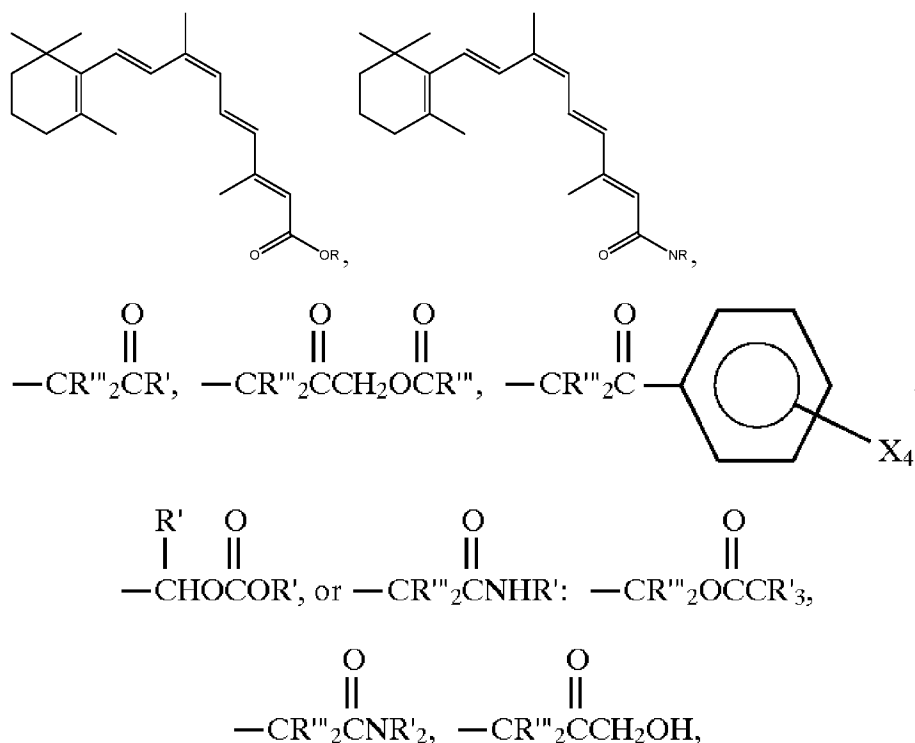


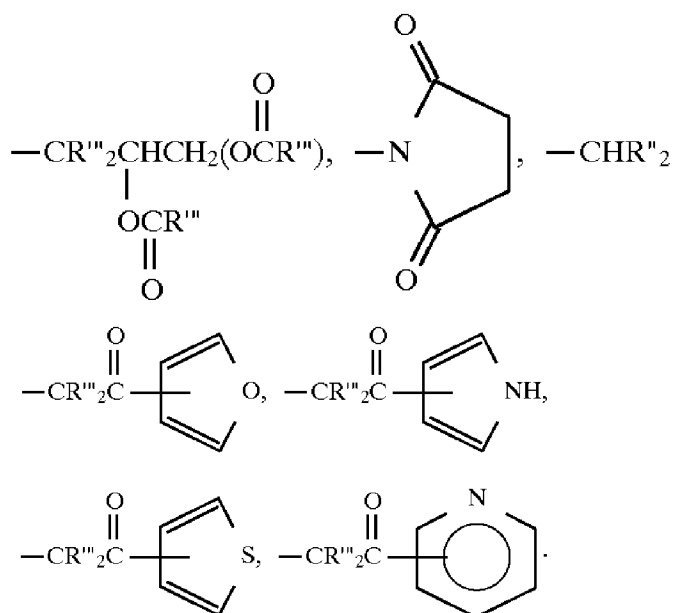
yl)nonatetraenoate (MDI 301):

(an RAR α – agonist

(see, e.g., U.S. Patent No. 5,837,728)), including salts, esters and prodrugs thereof.

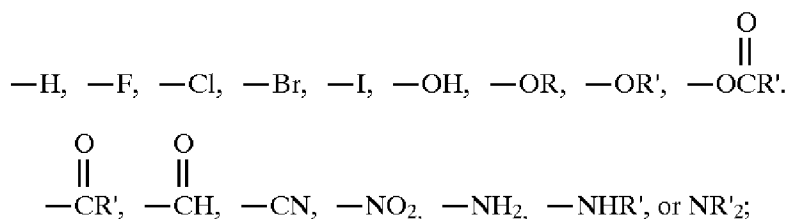
10 In certain embodiments, the *cis*-derivative of retinoic acid is any compound encompassed within one or more of the following formulas (see, e.g., U.S. Patent No. 5,837,728):





In some embodiments, such compounds encompassed within such formulas include salts, esters and prodrugs thereof.

5 In some embodiments, X is



In some embodiments, n is a number from 1 to 5.

In some embodiments, R' is H or any of the lower alkyls ranging from C₁ to C₆.

In some embodiments, R'' is selected from R', COOR', OR', and COR'.

10 In some embodiments, R''' is the hydrocarbon backbone of fatty acids.

In some embodiments, R'''' is R'' or the hydrocarbon backbone of fatty acids.

In some embodiments, R''''' is the lower alkyls ranging from C₁ to C₆.

In some embodiments, when there are two or more R', R'', R''', R'''', or R''''' groups attached to the same carbon, each R', R'', R''', R'''', or R''''' group may be the same as or different from the other R', R'', R''', R'''', or R''''' groups attached to that carbon.

15

Examples of such compounds encompassed within such formulas include, but are not limited to, 1-(9-cis-retinoyloxy)-2-propanone, 1-(9-cis-retinoyloxy)-3-decanoyloxy-2-propanone, 1,3-bis-(9-cis-retinoyloxy)-2-propanone, 1-(9-cis-retinoyloxy)-2-pinacolone, 2-(9-cis-retinoyloxy)-acetophenone, 9-cis-retinoyloxy methyl 2,2-dimethyl propanoate, 2-(9-

cis-retinoyloxy)-n-methyl-acetamide, 1-(9-cis-retinoyloxy)-3-hydroxy-2-propanone, 1-(9-cis-retinoyloxy)-2,3-dioleoylpropanone, and succinimidyl 9-cis-retinoate.

Such methods are not limited to treating, ameliorating, or preventing a particular condition involving myeloid leukemia cell growth. For example, in some embodiments, the condition involving myeloid leukemia cell growth is promyelocytic leukemia (PML) and/or other forms of myeloid leukemia (e.g., acute myeloid leukemia (AML), chronic myeloid leukemia, and myelodysplastic anemia). In some embodiments, the condition is a hematological cancer selected from T and B cell leukemias / lymphomas, and plasmacytomas / myelomas.

In some embodiments, such methods further comprise administering to the patient one or more anticancer agents. Such methods are not limited to particular anticancer agents. In some embodiments, the anticancer agent is a chemotherapeutic agent and/or a radiation therapy.

In certain embodiments, the present invention provides kits comprising a pharmaceutical composition comprising a *cis*-derivative of retinoic acid (e.g., MDI 301), and instructions for administering the pharmaceutical composition to a patient (e.g., a human patient) diagnosed with AML and/or PML. In some embodiments, the kits further comprise one or more anticancer agents (e.g., a chemotherapy agent). In some embodiments, the pharmaceutical composition comprising the *cis*-derivative of retinoic acid (e.g., MDI 301) does not induce a cytokine response, an inflammatory response, and/or systemic toxicity in the patient. In some embodiments, the pharmaceutical composition comprising the *cis*-derivative of retinoic acid (e.g., MDI 301) does not induce retinoic acid syndrome in the patient.

In certain embodiments, the present invention provides methods for inhibiting myeloid leukemia cell growth, comprising exposing to a sample (e.g., a sample from a human) comprising proliferating myeloid leukemia cells a composition comprising a *cis*-derivative of retinoic acid (e.g., MDI 301), wherein the exposing results in inhibition of myeloid leukemia cell growth. In some embodiments, the sample is from a human patient diagnosed with promyelocytic leukemia (PML) and/or other forms of myeloid leukemia (e.g., AML). In some embodiments, the sample comprises in vivo cells, ex vivo cells, and/or in vitro cells.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A-B: Effects of ATRA and MDI 301 on growth of myeloid leukemia cells *in vitro*. A. Concentration-dependence. Each of the four cell types was plated at 1×10^5 cells per mL in growth medium and treated for three days with the indicated concentration of ATRA or MDI 301. At the end of the treatment phase, cells were harvested and counted. Values shown are means and standard deviations based on 3-5 separate experiments (NB4, HL60 and K562) with duplicate or triplicate samples per data point in each experiment. Data for OCI-M2 cells are based on duplicate experiments. Data were analyzed by ANOVA followed by paired-group comparison. Asterisk indicates statistical difference from control at $p < 0.05$. B. Time-response. HL60 cells were treated for 1-3 days with 15 μ M ATRA or MDI 301. At each time-point, cells were harvested and counted. Values shown are means and standard deviations based on one experiment with triplicate samples per data point. Data were analyzed by ANOVA followed by paired-group comparison. Asterisk indicates statistical difference from control with both ATRA and MDI 301 at $p < 0.05$. The experiment was repeated three times with similar results.

FIG. 2: Effects of ATRA and MDI 301 on attachment of myeloid leukemia cells in culture. Cells were incubated for 4 days. At the end of the treatment phase, non-attached cells were removed and attached cells fixed in 10% buffered formalin. Cells were counted microscopically. Values shown are means and standard deviations (percent attached) based on one experiment with five samples per data point. Data were analyzed by ANOVA followed by paired-group comparison. *indicates $p < 0.05$ relative to control. The experiment was repeated three times with similar results. Insert: NB4 cells.

FIG. 3: Effects of ATRA and MDI 301 on growth of HL60 xenograft in athymic mice. Mice were injected with HL60 cells by the subcutaneous route on day-zero and then treated with ATRA or MDI 301 (20 mg/kg) on a three-day schedule. Tumor volumes were assessed as described in the Materials and Methods section. Values shown are tumor volumes of individual mice at days-7, 15 and 25. Insert: Gross and histological comparison of tumors in control mice and mice treated with either ATRA or MDI 301. Arrows in the gross images identify tumors. Other than size, there is little to distinguish the tumors between the treatment groups. At the histological level (hematoxylin and eosin - stained, tumors in all three groups appear as sheets of undifferentiated cells.

FIG. 4: Combined skin irritation score. Each animal was scored for skin irritation on each day throughout the treatment phase. Parameters included redness/edema,

dryness/cracking and flaking. Each parameter was scored between 0 and 4+. Individual values from each animal in each group were combined to give a single score for each treatment group for each day. Data are means based on n=14, n=7 and n=8 mice for control, ATRA and MDI 301 groups, respectively. Irritation scores from all days were averaged, and examined statistically. Mice in both retinoid-treated groups were statistically different from control and ATRA-treated mice were statistically higher than MDI 301 – treated animals (P<0.01 for both retinoids versus control and for ATRA versus MDI 301).

FIG. 5: Cytokine elaboration. Each data point represents cytokine level in serum from individual mice obtained at euthanasia. With IL-6 and KC, differences between ATRA and control were significant at p<0.05 by ANOVA followed by paired group comparisons. In no case was the average cytokine level in sera from MDI 301 – treated statistically higher than average control level.

DEFINITIONS

To facilitate an understanding of the invention, the following terms have the meanings defined below.

The term "host cell" or "cell" refers to any cell which is used in any of the methods of the present invention and may include prokaryotic cells, eukaryotic cells, yeast cells, bacterial cells, plant cells, animal cells, such as, reptilian cells, bird cells, fish cells, mammalian cells.

Preferred cells include those derived from humans, dogs, cats, horses, cattle, sheep, pigs, llamas, gerbils, squirrels, goats, bears, chimpanzees, mice, rats, rabbits, etc. The term cells includes transgenic cells from cultures or from transgenic organisms. The cells may be from a specific tissue, body fluid, organ (e.g., brain tissue, nervous tissue, muscle tissue, retina tissue, kidney tissue, liver tissue, etc.), or any derivative fraction thereof. The term includes healthy cells, transgenic cells, cells affected by internal or exterior stimuli, cells suffering from a disease state or a disorder, cells undergoing transition (e.g., mitosis, meiosis, apoptosis, etc.), etc. The term also refers to cells *in vivo* or *in vitro* (e.g., the host cell may be located in a transgenic animal or in a human subject).

As used herein, the terms "host" and "subject" refer to any animal, including but not limited to, human and non-human animals (e.g. rodents, arthropods, insects (e.g., Diptera), fish (e.g., zebrafish), non-human primates, ovines, bovines, ruminants, lagomorphs, porcines, caprines, equines, canines, felines, aves, etc.), that is studied, analyzed, tested, diagnosed or treated. As used herein, the terms "host" and "subject" are used interchangeably.

The term “pharmaceutical composition” refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*.

The term “pharmaceutically acceptable carrier” refers to any of the standard
5 pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants. (*See e.g.*, Martin, Remington's Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, PA [1975]).

10 The term “pharmaceutically acceptable salt” refers to any pharmaceutically acceptable salt (*e.g.*, acid or base) of a compound of the present invention which, upon administration to a subject, is capable of providing a compound of this invention or an active metabolite or residue thereof. As is known to those of skill in the art, “salts” of the compounds of the present invention may be derived from inorganic or organic acids and
15 bases. Examples of acids include, but are not limited to, hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycolic, lactic, salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, ethanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic, benzenesulfonic acid, and the like. Other acids, such as oxalic, while sometime not in themselves pharmaceutically acceptable, may be employed in the
20 preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts. Examples of bases include alkali metals (*e.g.*, sodium) hydroxides, alkaline earth metals (*e.g.*, magnesium), hydroxides, ammonia, and compounds of formula NW_4^+ , wherein W is C_{1-4} alkyl, and the like. Examples of salts include, but are not limited to, acetate, adipate, alginate, aspartate, benzoate,
25 benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, flucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, palmoate, pectinate, persulfate, phenylpropionate,
30 picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, undecanoate, and the like. Other examples of salts include anions of the compounds of the present invention compounded with a suitable cation such as Na^+ , NH_4^+ , and NW_4^+ (wherein W is a C_{1-4} alkyl group), and the like.

For therapeutic use, salts of the compounds of the present invention are contemplated as being pharmaceutically acceptable. However, salts of acids and bases that are non-pharmaceutically acceptable may also find use, for example, in the preparation or purification of a pharmaceutically acceptable compound.

5 The term “effective amount” refers to the amount of a compound sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not limited or intended to be limited to a particular formulation or administration route.

10 The term “second agent” refers to a therapeutic agent other than the *cis*-derivative of retinoic acid (e.g., MDI 301) in accordance with the present invention. In certain instances, the second agent is an anti-proliferative agent.

15 The term “co-administration” refers to the administration of at least two agent(s) (e.g., a *cis*-derivative of retinoic acid (e.g., MDI 301)) or therapies to a subject. In some embodiments, the co-administration of two or more agents/therapies is concurrent. In other embodiments, a first agent/therapy is administered prior to a second agent/therapy. Those of skill in the art understand that the formulations and/or routes of administration of the various agents/therapies used may vary. The appropriate dosage for co-administration can be readily determined by one skilled in the art. In some embodiments, when agents/therapies are co-administered, the respective agents/therapies are administered at lower dosages than
20 appropriate for their administration alone. Thus, co-administration is especially desirable in embodiments where the co-administration of the agents/therapies lowers the requisite dosage of a known potentially harmful (e.g., toxic) agent(s).

25 The term “combination therapy” includes the administration of a *cis*-derivative of retinoic acid (e.g., MDI 301) and at least a second agent as part of a specific treatment regimen intended to provide the beneficial effect from the co-action of these therapeutic agents. The beneficial effect of the combination includes, but is not limited to, pharmacokinetic or pharmacodynamic co-action resulting from the combination of therapeutic agents. Administration of these therapeutic agents in combination typically is carried out over a defined time period (usually minutes, hours, days or weeks depending upon
30 the combination selected). “Combination therapy” may, but generally is not, intended to encompass the administration of two or more of these therapeutic agents as part of separate monotherapy regimens that incidentally and arbitrarily result in the combinations of the present invention. “Combination therapy” is intended to embrace administration of these therapeutic agents in a sequential manner, that is, wherein each therapeutic agent is

administered at a different time, as well as administration of these therapeutic agents, or at least two of the therapeutic agents, in a substantially simultaneous manner. Substantially simultaneous administration can be accomplished, for example, by administering to the subject a single capsule having a fixed ratio of each therapeutic agent or in multiple, single capsules for each of the therapeutic agents. Sequential or substantially simultaneous administration of each therapeutic agent can be effected by any appropriate route including, but not limited to, oral routes, intravenous routes, intramuscular routes, and direct absorption through mucous membrane tissues. The therapeutic agents can be administered by the same route or by different routes. For example, a first therapeutic agent of the combination selected may be administered by intravenous injection while the other therapeutic agents of the combination may be administered orally. Alternatively, for example, all therapeutic agents may be administered orally or all therapeutic agents may be administered by intravenous injection. The sequence in which the therapeutic agents are administered is not narrowly critical. "Combination therapy" also can embrace the administration of the therapeutic agents as described above in further combination with other biologically active ingredients and non-drug therapies (e.g., surgery or radiation treatment.) Where the combination therapy further comprises a non-drug treatment, the non-drug treatment may be conducted at any suitable time so long as a beneficial effect from the co-action of the combination of the therapeutic agents and non-drug treatment is achieved. For example, in appropriate cases, the beneficial effect is still achieved when the non-drug treatment is temporally removed from the administration of the therapeutic agents, perhaps by days or even weeks.

DETAILED DESCRIPTION

Retinoids regulate growth and differentiation in the epithelium and have demonstrated anti-proliferative activity in several tumor types (see, e.g., Bollag W, et al., *Ann Oncol.* 1992; 3(7):513-26; Smith MA, et al., *J. Clin Oncol* 1992; 10: 839-864; Altucci L, et al., *Nat Rev Drug Discov.* 2007; 6(10):793-810; Tang /x-H, et al., *Ann. Rev. Pathol Mech. Dis.* 2011; 6:345-364). At present, 13-cis retinoic acid is used clinically in high-risk neuroblastoma (see, e.g., Reynolds CP, et al., *Cancer Lett.* 2003;197(1-2):185-92) and all-trans retinoic acid (ATRA) is used in promyelocytic leukemia (PML) (see, e.g., Huang ME, et al., *Blood.* 1988;72(2):567-72; Fenaux P, et al., *European APL Group. Lancet.* 1994;343(8904):1033; Sanz MA, et al., *Blood.* 2010;115(25):5137-46; Lo-Coco F, et al., *N Engl J Med.* 2013;369(2):111-21; Wang G, et al., *Hematol Oncol.* 2004;22(2):63-71; Shen ZX, et al., *Proc Natl Acad Sci USA.* 2004;101(15):5328-35). In approximately 90% of patients with PML,

all-trans retinoic acid (ATRA) is effective owing to a translocation of the PML gene onto the retinoic acid receptor – α (RAR α) gene, and suppression of transcriptional activity of the PML-RAR α complex (see, e.g., Kakizuka A, et al., *Cell*. 1991;66(4):663–674; Borrow J, et al., *Science*. 1990;249(4976):1577-80; de Thé H, et al., *Nature*. 1990;347(6293):558-61).

5 ATRA induces PML cells to differentiate into mature granulocytic cells, resulting in growth inhibition and apoptosis (see, e.g., Barber N, et al., *Leuk Res*. 2008;32(2):315-22; Castaigne S, et al., *Blood*. 1990;76(9):1704-9; Degos L, et al., *Blood*. 1995;85(10):2643-53).

A limitation of retinoid therapy (alone or in combination with other agents) is retinoic acid syndrome (see, e.g., Frankel SR, et al., *Ann Intern Med*. 1992;117:292-296; Fenaux P, et al., *Drug Safety*. 1998;18(4):273-9; Larson RS, et al., *Best Pract Res Clin Haematol*. 2003;16(3):453-61) – a side effect characterized by sudden onset of fever, chills, and respiratory distress. Edema and weight gain, pleural or pericardial effusions, pulmonary hemorrhage, and episodic hypotension as well as kidney failure can all occur. These symptoms can appear anywhere from 2 to 21 days post ATRA treatment. Based on early
15 studies in PML, incidence rates of up to 25-30% were reported, with fatality rates of up to 30%. However, as the syndrome came to be more readily recognized and treated earlier, the reported incidence has increased while serious consequences including fatality rate have declined (see, e.g., Vahdat L, et al., *Blood*. 1994;84(11):3843-9; De Botton S, et al., *Blood*. 1998;92(8):2712-8; Tallman MS, et al., *Blood*. 2000;95(1):90-5). Nonetheless, this
20 consequence of ATRA therapy is always a serious matter that can compromise therapy even if it can be controlled.

While the patho-physiology of retinoic acid syndrome is not completely understood, it is thought to reflect vascular leakage and hemorrhage, due to the rapid release of multiple bioactive mediators into the circulation (see, e.g., Gianni M, et al., *Eur Cytokine Netw*. 25 1995;6(3):157-65; Hsu HC, et al., *Eur J Haematol*. 1999;63(1):11-8) from the tumor cells that differentiate and, ultimately, undergo apoptosis. Additionally, many of the differentiating tumor cells acquire adhesive capacity (see, e.g., Marchetti M, et al., *Br J Haematol*. 1996;93(2):360-6; Brown DC, et al., *Br J Haematol*. 1999;107(1):86-98), which leads to “sludging” in the capillary beds and infiltration into the extravascular tissue spaces. While
30 mediators released from the tumor cells themselves are likely contributors to the syndrome, ATRA also affects host parenchymal elements including epithelial cells, fibroblasts and endothelial cells. Release of pro-inflammatory cytokines from these cells in the skin has been shown to occur in response to ATRA treatment (see, e.g., Varani J, et al., *Toxicol*

Pathol. 2007;35(5):693-701; Varani J, et al., Arch Dermatol Res. 2007;298(9):439-48). The skin irritation response in people treated topically with ATRA (i.e., Tretinoin or Renova) is thought to be due to an underlying inflammatory response – driven by parenchymal cell – generated pro-inflammatory cytokines. It is reasonable to hypothesize that the effects seen with systemic ATRA use in myeloid leukemia are due, in part at least, to a similar underlying mechanism – i.e., to release of pro-inflammatory mediators from host parenchymal cells as well as from the differentiating leukemic cells.

MDI 301 is a picolinate ester of 9-*cis* retinoic acid (see, e.g., U.S. Patent No. 5,837,728). Studies conducted in rodents (see, e.g., Varani J, et al., Arch. Dermatol. Res. 2003;295(6):255-62; Warner RL, et al., Wound Repair Regen. 2008;16(1):117-24), Gottingen minipigs (see, e.g., Dame MK, et al., In Vitro Cell Dev Biol Anim. 2009;45(9):551-7), and human skin organ culture (see, e.g., Varani J, et al., Arch Dermatol Res. 2007;298(9):439-48; Zeng W, et al., J Diabetes Complications. 2011;25(6):398-404) have demonstrated that MDI 301 is similar to ATRA in regard to skin-repair efficacy but induces much less skin irritation. In human skin organ culture, MDI 301 does not up-regulate pro-inflammatory cytokines nor alter adhesion factor expression, in contrast to what is seen with ATRA (see, e.g., Varani J, et al., Arch Dermatol Res. 2007;298(9):439-48; Varani J, et al., Toxicol Pathol. 2007;35(5):693-701).

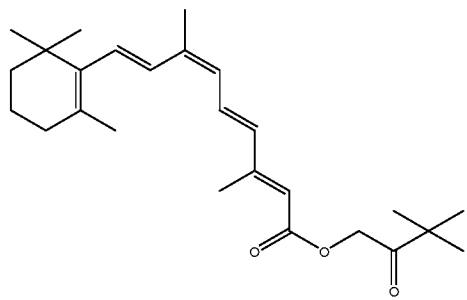
Given the lack of a pro-inflammatory response to MDI 301, experiments conducted during the course of developing embodiments for the present invention compared MDI 301 with ATRA for effects on a series of human myeloid leukemia cell lines in vitro and in vivo. A goal was to demonstrate that MDI 301 had efficacy comparable to that of ATRA but with reduced toxicity. The results demonstrated improved efficacy and reduced toxicity for MDI 301 in comparison with ATRA.

Accordingly, methods of treatment using inhibitors of myeloid leukemia cell growth are provided. Such methods include, but are not limited to, methods of treating myeloid leukemia (e.g., promyelocytic leukemia, acute myeloid leukemia). Such inhibitors of myeloid leukemia cell growth include, but are not limited to, pharmaceutical compositions including a *cis*-derivative(s) of retinoic acid (e.g., pinacolyl 9-*cis*-retinoate, (2E, 4E, 6Z, 8E)-3,3-Dimethyl-2-oxo-1-butyl 3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nonatetraenoate (MDI 301)). Related kits and compositions are further provided.

As used herein, an "inhibitor of myeloid leukemia cell growth" is a compound that inhibits (e.g., hinders, prevents, interferes with, etc.) myeloid leukemia cell growth. In some embodiments, the inhibitor of myeloid leukemia cell growth not only inhibits growth of

myeloid leukemia cells, but further causes differentiation and/or apoptosis of such cells. In some embodiments, the inhibitor of myeloid leukemia cell growth is a *cis*-derivative of retinoic acid.

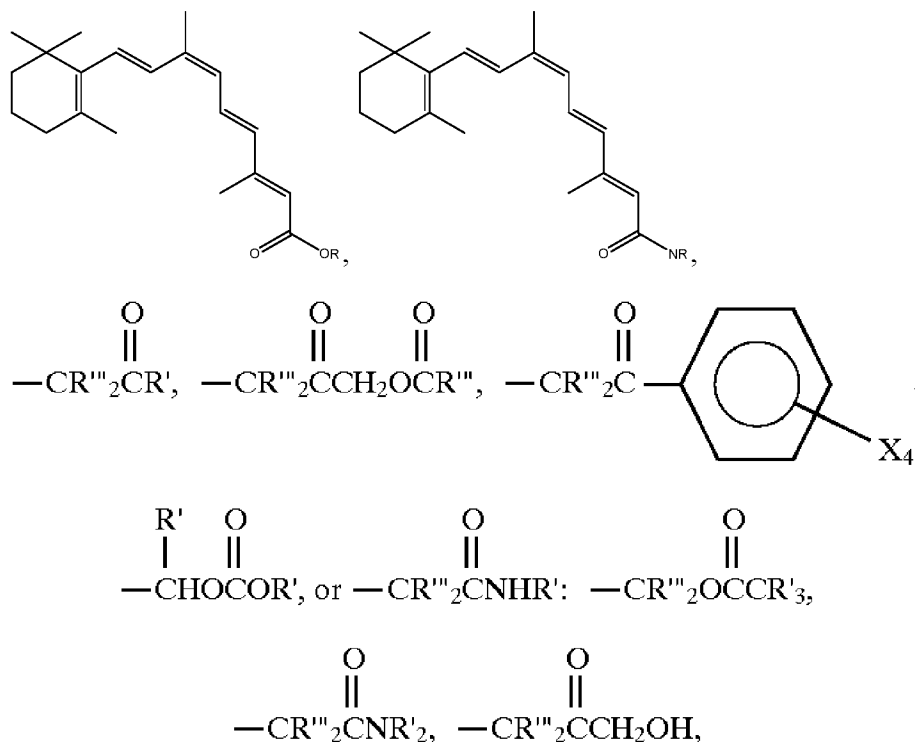
In certain embodiments, the inhibitor of myeloid leukemia cell growth is pinacolyl 9-
5 *cis*-retinoate, (2E, 4E, 6Z, 8E)-3,3-Dimethyl-2-oxo-1-butyl 3,7-dimethyl-9-(2,6,6-

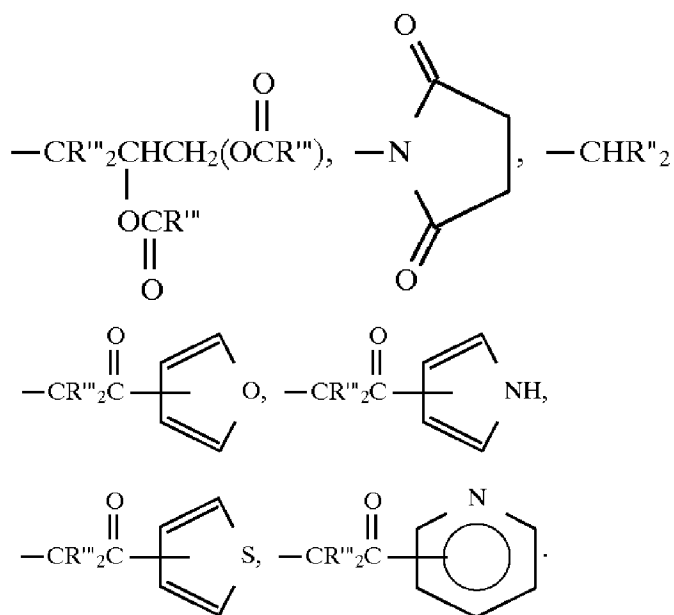


trimethylcyclohexen-1-yl)nonatetraenoate (MDI 301):

including salts, esters and prodrugs thereof (see, e.g., U.S. Patent No. 5,837,728).

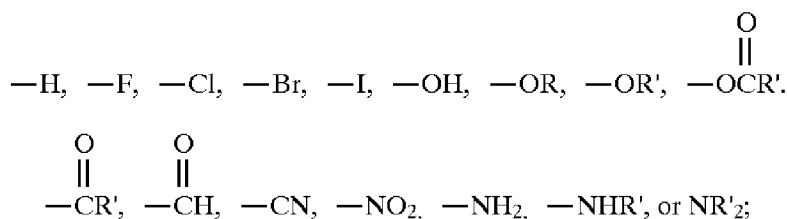
In certain embodiments, the inhibitor of myeloid leukemia cell growth is any
10 compound encompassed within one or more of the following formulas (see, e.g., U.S. Patent No. 5,837,728):





In some embodiments, such compounds encompassed within such formulas include salts, esters and prodrugs thereof.

5 In some embodiments, X is



In some embodiments, n is a number from 1 to 5.

In some embodiments, R' is H or any of the lower alkyls ranging from C₁ to C₆.

In some embodiments, R'' is selected from R', COOR', OR', and COR'.

10 In some embodiments, R''' is the hydrocarbon backbone of fatty acids.

In some embodiments, R'''' is R'' or the hydrocarbon backbone of fatty acids.

In some embodiments, R''''' is the lower alkyls ranging from C₁ to C₆.

In some embodiments, when there are two or more R', R'', R''', R'''', or R''''' groups attached to the same carbon, each R', R'', R''', R'''', or R''''' group may be the same
15 as or different from the other R', R'', R''', R'''', or R''''' groups attached to that carbon.

Examples of such compounds encompassed within such formulas include, but are not limited to, 1-(9-cis-retinoyloxy)-2-propanone, 1-(9-cis-retinoyloxy)-3-decanoyloxy-2-propanone, 1,3-bis-(9-cis-retinoyloxy)-2-propanone, 1-(9-cis-retinoyloxy)-2-pinacolone, 2-(9-cis-retinoyloxy)-acetophenone, 9-cis-retinoyloxy methyl 2,2-dimethyl propanoate, 2-(9-

cis-retinoyloxy)-n-methyl-acetamide, 1-(9-cis-retinoyloxy)-3-hydroxy-2-propanone, 1-(9-cis-retinoyloxy)-2,3-dioleoylpropanone, and succinimidyl 9-cis-retinoate.

In certain embodiments, *cis*-derivatives of retinoic acid represent a significant improvement over *trans*-derivatives of retinoic acid in terms of inhibiting myeloid leukemia cell growth. Indeed, in experiments conducted during the course of developing embodiments for the present invention, MDI 301 was shown to suppress growth of several human myeloid leukemia cell lines (e.g., HL60, NB4, OCI-M2 and K562) *in vitro* and slow the growth of xenografted tumor growth (HL60) in athymic nude mice. *In vitro*, MDI 301 was shown to be comparable to all-*trans* retinoic acid (ATRA) while *in vivo*, MDI 301 was slightly more efficacious than ATRA. Unlike what was seen with ATRA treatment, MDI 301 did not induce a cytokine response in the treated animals and the severe inflammatory changes and systemic toxicity typically seen with ATRA did not occur.

The inhibitors of myeloid leukemia cell growth (e.g., MDI 301) described in the present invention are useful in the preparation of pharmaceutical formulation, also synonymously referred to herein as “medicaments,” to treat a variety of conditions associated with myeloid leukemia cell growth (e.g., myeloid leukemia) (e.g., PML, AML). In addition, the inhibitors of myeloid leukemia cell growth (e.g., MDI 301) are also useful for preparing medicaments for treating other disorders wherein the effectiveness of such inhibitors are known or predicted. Such disorders include, but are not limited to, other types of cancer related disorders. The methods and techniques for preparing medicaments of an inhibitor of myeloid leukemia cell growth (e.g., MDI 301) described in the present invention are well-known in the art. Exemplary pharmaceutical formulations and routes of delivery are described below.

One of skill in the art will appreciate that any one or more of the inhibitors of myeloid leukemia cell growth (e.g., MDI 301) described herein, including the many specific embodiments, are prepared by applying standard pharmaceutical manufacturing procedures. Such pharmaceutical formulations can be delivered to the subject by using delivery methods that are well-known in the pharmaceutical arts.

In some embodiments of the present invention, the compositions are administered alone, while in some other embodiments, the compositions are preferably present in a pharmaceutical formulation comprising at least one active ingredient/agent, as defined above, together with a solid support or alternatively, together with one or more pharmaceutically acceptable carriers and optionally other therapeutic agents. Each carrier must be “acceptable”

in the sense that it is compatible with the other ingredients of the formulation and not injurious to the subject.

Contemplated formulations include those suitable oral, rectal, nasal, topical (including transdermal, buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous and intradermal) and pulmonary administration. In some 5
embodiments, formulations are conveniently presented in unit dosage form and are prepared by any method known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing 10
into association (e.g., mixing) the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, wherein each preferably contains a predetermined amount of the active ingredient; as a powder or granules; as a 15
solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. In other embodiments, the active ingredient is presented as a bolus, electuary, or paste, etc.

In some embodiments, tablets comprise at least one active ingredient and optionally one or more accessory agents/carriers are made by compressing or molding the respective 20
agents. In some embodiments, compressed tablets are prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g., povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g., sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Molded 25
tablets are made by molding in a suitable machine a mixture of the powdered compound (e.g., active ingredient) moistened with an inert liquid diluent. Tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric 30
coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or

sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Pharmaceutical compositions for topical administration according to the present invention are optionally formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils. In alternative embodiments, topical formulations comprise patches or dressings such as a bandage or adhesive plasters impregnated with active ingredient(s), and optionally one or more excipients or diluents. In some embodiments, the topical formulations include a compound(s) that enhances absorption or penetration of the active agent(s) through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethylsulfoxide (DMSO) and related analogues.

If desired, the aqueous phase of a cream base includes, for example, at least about 30% w/w of a polyhydric alcohol, i.e., an alcohol having two or more hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol and mixtures thereof.

In some embodiments, oily phase emulsions of this invention are constituted from known ingredients in a known manner. This phase typically comprises a lone emulsifier (otherwise known as an emulgent), it is also desirable in some embodiments for this phase to further comprise a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil.

Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier so as to act as a stabilizer. In some embodiments it is also preferable to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and/or fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glyceryl monostearate and sodium lauryl sulfate.

The choice of suitable oils or fats for the formulation is based on achieving the desired properties (e.g., cosmetic properties), since the solubility of the active compound/agent in most oils likely to be used in pharmaceutical emulsion formulations is very low. Thus creams should preferably be a non-greasy, non-staining and washable products with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl

palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

5 Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the agent.

 Formulations for rectal administration may be presented as a suppository with suitable base comprising, for example, cocoa butter or a salicylate. Likewise, those for vaginal
10 administration may be presented as pessaries, creams, gels, pastes, foams or spray formulations containing in addition to the agent, such carriers as are known in the art to be appropriate.

 Formulations suitable for nasal administration, wherein the carrier is a solid, include coarse powders having a particle size, for example, in the range of about 20 to about 500
15 microns which are administered in the manner in which snuff is taken, i.e., by rapid inhalation (e.g., forced) through the nasal passage from a container of the powder held close up to the nose. Other suitable formulations wherein the carrier is a liquid for administration include, but are not limited to, nasal sprays, drops, or aerosols by nebulizer, and include aqueous or oily solutions of the agents.

20 Formulations suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to
25 target the compound to blood components or one or more organs. In some embodiments, the formulations are presented/formulated in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be
30 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 subdose, as herein above-recited, or an appropriate fraction thereof, of an agent. It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of

formulation in question, for example, those suitable for oral administration may include such further agents as sweeteners, thickeners and flavoring agents. It also is intended that the agents, compositions and methods of this invention be combined with other suitable compositions and therapies. Still other formulations optionally include food additives
5 (suitable sweeteners, flavorings, colorings, etc.), phytonutrients (e.g., flax seed oil), minerals (e.g., Ca, Fe, K, etc.), vitamins, and other acceptable compositions (e.g., conjugated linoelic acid), extenders, and stabilizers, etc.

In some embodiments, the inhibitors of myeloid leukemia cell growth (e.g., MDI 301) described in the present invention are provided in unsolvated form or are in non-aqueous
10 solutions (e.g., ethanol). The inhibiting agents may be generated to allow such formulations through the production of specific crystalline polymorphs compatible with the formulations.

In certain embodiments, the present invention provides instructions for administering said inhibitors of myeloid leukemia cell growth (e.g., MDI 301) to a subject. In certain embodiments, the present invention provides instructions for using the compositions
15 contained in a kit for the treatment of conditions characterized by the dysregulation of apoptotic processes in a cell or tissue (e.g., providing dosing, route of administration, decision trees for treating physicians for correlating patient-specific characteristics with therapeutic courses of action). In certain embodiments, the present invention provides instructions for using the compositions contained in the kit to treat a variety of medical
20 conditions associated with myeloid leukemia cell growth (e.g., AML, PML, chronic myeloid leukemia, and myelodysplastic anemia). In certain embodiments, the present invention provides instructions for using the compositions contained in the kit to treat a variety of medical conditions associated with a hematological cancer selected from T and B cell leukemias / lymphomas, and plasmacytomas / myelomas.

Various delivery systems are known and can be used to administer therapeutic agents
25 (e.g., the inhibitors of myeloid leukemia cell growth (e.g., MDI 301) described in the present invention) of the present invention, e.g., encapsulation in liposomes, microparticles, microcapsules, receptor-mediated endocytosis, and the like. Methods of delivery include, but are not limited to, intra-arterial, intra-muscular, intravenous, intranasal, and oral routes. In
30 specific embodiments, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, injection, or by means of a catheter.

It is contemplated that the agents identified can be administered to subjects or individuals having, susceptible to or at risk of developing pathological growth of target cells and correlated conditions. When the agent is administered to a subject such as a mouse, a rat or a human patient, the agent can be added to a pharmaceutically acceptable carrier and systemically or topically administered to the subject. To determine patients that can be beneficially treated, a tissue sample is removed from the patient and the cells are assayed for sensitivity to the agent.

Therapeutic amounts are empirically determined and vary with the pathology being treated, the subject being treated and the efficacy and toxicity of the agent. When delivered to an animal, the method is useful to further confirm efficacy of the agent.

In some embodiments, *in vivo* administration is effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations are carried out with the dose level and pattern being selected by the treating physician.

Suitable dosage formulations and methods of administering the agents are readily determined by those of skill in the art. Preferably, the inhibitors of myeloid leukemia cell growth (e.g., MDI 301) provided herein are administered at about 0.01 mg/kg to about 200 mg/kg, more preferably at about 0.1 mg/kg to about 100 mg/kg, even more preferably at about 0.5 mg/kg to about 50 mg/kg. When the inhibitors of myeloid leukemia cell growth (e.g., MDI 301) described herein are co-administered with another agent (e.g., as sensitizing agents), the effective amount may be less than when the agent is used alone.

The pharmaceutical compositions can be administered orally, intranasally, parenterally or by inhalation therapy, and may take the form of tablets, lozenges, granules, capsules, pills, ampoules, suppositories or aerosol form. They may also take the form of suspensions, solutions and emulsions of the active ingredient in aqueous or non-aqueous diluents, syrups, granulates or powders. In addition to a CK2 inhibiting agent described in the present invention, the pharmaceutical compositions can also contain other pharmaceutically active compounds or a plurality of Inhibitors of myeloid leukemia cell growth (e.g., MDI 301) described in the present invention.

More particularly, an agent of the present invention also referred to herein as the active ingredient, may be administered for therapy by any suitable route including, but not limited to, oral, rectal, nasal, topical (including, but not limited to, transdermal, aerosol,

buccal and sublingual), vaginal, parental (including, but not limited to, subcutaneous, intramuscular, intravenous and intradermal) and pulmonary. It is also appreciated that the preferred route varies with the condition and age of the recipient, and the disease being treated.

5 Ideally, the agent should be administered to achieve peak concentrations of the active compound at sites of disease. This may be achieved, for example, by the intravenous injection of the agent, optionally in saline, or orally administered, for example, as a tablet, capsule or syrup containing the active ingredient.

10 Desirable blood levels of the agent may be maintained by a continuous infusion to provide a therapeutic amount of the active ingredient within disease tissue. The use of operative combinations is contemplated to provide therapeutic combinations requiring a lower total dosage of each component antiviral agent than may be required when each individual therapeutic compound or drug is used alone, thereby reducing adverse effects.

15 The present invention also includes methods involving co-administration of the inhibitors of myeloid leukemia cell growth (e.g., MDI 301) described herein with one or more additional active agents. Indeed, it is a further aspect of this invention to provide methods for enhancing prior art therapies and/or pharmaceutical compositions by co-administering an inhibitor of myeloid leukemia cell growth (e.g., MDI 301) described herein. In co-administration procedures, the agents may be administered concurrently or sequentially.
20 In one embodiment, the inhibitors of myeloid leukemia cell growth (e.g., MDI 301) described herein are administered prior to the other active agent(s). The pharmaceutical formulations and modes of administration may be any of those described above. In addition, the two or more co-administered chemical agents, biological agents or radiation may each be administered using different modes or different formulations.

25 The agent or agents to be co-administered depends on the type of condition being treated. For example, when the condition being treated is a type of cancer involving myeloid leukemia cell growth (e.g., PML, AML, T and B cell leukemias / lymphomas, myelodysplastic anemia, and plasmacytoma/multiple myeloma), the additional agent can be a chemotherapeutic antineoplastic, and/or a apoptosis-modulating agent. The additional agents
30 to be co-administered can be any of the well-known agents in the art for a particular disorder, including, but not limited to, those that are currently in clinical use and/or experimental use.

 In certain embodiments, the present invention provides methods (e.g., therapeutic applications) for treating conditions associated with myeloid leukemia cell growth. The present invention is not limited to a particular type of method. In some embodiments, the

methods for treating conditions associated with myeloid leukemia cell growth comprise a) providing: i. target cells having growing myeloid leukemia cells; and ii. a composition (e.g., a composition comprising an inhibitor of myeloid leukemia cell growth); and b) exposing the target cells to the composition under conditions such that the exposure results in inhibition of myeloid leukemia cell growth.

The methods are not limited to treating a particular condition associated with myeloid leukemia cell growth. In some embodiments, the condition associated with myeloid leukemia cell growth is a type of cancer involving myeloid leukemia cell growth. In some embodiments, the condition is AML. In some embodiments, the condition is PML. In some embodiments, the condition is chronic myeloid leukemia. In some embodiments, the condition is myelodysplastic anemia. In some embodiments, the condition is a hematological cancer selected from T and B cell leukemias / lymphomas, and plasmacytomas / myelomas.

In some embodiments, the target cells are within a living mammal (e.g., human, horse, dog, cat, pig, rat, mouse, ape, monkey).

Additionally, any one or more of these compounds can be used in combination with at least one other therapeutic agent. In a particular embodiment, the additional therapeutic agent(s) is an anticancer agent.

A number of suitable anticancer agents are contemplated for use in the methods of the present invention. Indeed, the present invention contemplates, but is not limited to, administration of numerous anticancer agents such as: agents that induce apoptosis; polynucleotides (e.g., anti-sense, ribozymes, siRNA); polypeptides (e.g., enzymes and antibodies); biological mimetics; alkaloids; alkylating agents; antitumor antibiotics; antimetabolites; hormones; platinum compounds; monoclonal or polyclonal antibodies (e.g., antibodies conjugated with anticancer drugs, toxins, defensins), toxins; radionuclides; biological response modifiers (e.g., interferons (e.g., IFN- α) and interleukins (e.g., IL-2)); adoptive immunotherapy agents; hematopoietic growth factors; gene therapy reagents (e.g., antisense therapy reagents and nucleotides); tumor vaccines; angiogenesis inhibitors; proteasome inhibitors; NF-KB modulators; anti-CDK compounds; HDAC inhibitors; and the like. Numerous other examples of chemotherapeutic compounds and anticancer therapies suitable for co-administration with the disclosed compounds are known to those skilled in the art.

In certain embodiments, anticancer agents comprise agents that induce or stimulate apoptosis. Agents that induce apoptosis include, but are not limited to, radiation (e.g., X-rays, gamma rays, UV); tumor necrosis factor (TNF)-related factors (e.g., TNF family receptor

proteins, TNF family ligands, TRAIL, antibodies to TRAIL-R1 or TRAIL-R2); kinase inhibitors (*e.g.*, epidermal growth factor receptor (EGFR) kinase inhibitor, vascular growth factor receptor (VGFR) kinase inhibitor, fibroblast growth factor receptor (FGFR) kinase inhibitor, platelet-derived growth factor receptor (PDGFR) kinase inhibitor, and Bcr-Abl kinase inhibitors (such as GLEEVEC)); antisense molecules; antibodies (*e.g.*, HERCEPTIN, RITUXAN, ZEVALIN, and AVASTIN); anti-estrogens (*e.g.*, raloxifene and tamoxifen); anti-androgens (*e.g.*, flutamide, bicalutamide, finasteride, aminoglutethamide, ketoconazole, and corticosteroids); cyclooxygenase 2 (COX-2) inhibitors (*e.g.*, celecoxib, meloxicam, NS-398, and non-steroidal anti-inflammatory drugs (NSAIDs)); anti-inflammatory drugs (*e.g.*, butazolidin, DECADRON, DELTASONE, dexamethasone, dexamethasone intensol, DEXONE, HEXADROL, hydroxychloroquine, METICORTEN, ORADEXON, ORASONE, oxyphenbutazone, PEDIAPRED, phenylbutazone, PLAQUENIL, prednisolone, prednisone, PRELONE, and TANDEARIL); and cancer chemotherapeutic drugs (*e.g.*, irinotecan (CAMPTOSAR), CPT-11, fludarabine (FLUDARA), dacarbazine (DTIC), dexamethasone, mitoxantrone, MYLOTARG, VP-16, cisplatin, carboplatin, oxaliplatin, 5-FU, doxorubicin, gemcitabine, bortezomib, gefitinib, bevacizumab, TAXOTERE or TAXOL); cellular signaling molecules; ceramides and cytokines; staurosporine, and the like.

In still other embodiments, the compositions and methods of the present invention provide a compound of the invention and at least one anti-hyperproliferative or antineoplastic agent selected from alkylating agents, antimetabolites, and natural products (*e.g.*, herbs and other plant and/or animal derived compounds).

Alkylating agents suitable for use in the present compositions and methods include, but are not limited to: 1) nitrogen mustards (*e.g.*, mechlorethamine, cyclophosphamide, ifosfamide, melphalan (L-sarcosine); and chlorambucil); 2) ethylenimines and methylmelamines (*e.g.*, hexamethylmelamine and thiotepa); 3) alkyl sulfonates (*e.g.*, busulfan); 4) nitrosoureas (*e.g.*, carmustine (BCNU); lomustine (CCNU); semustine (methyl-CCNU); and streptozocin (streptozotocin)); and 5) triazenes (*e.g.*, dacarbazine (DTIC); dimethyltriazenoimid-azolecarboxamide).

In some embodiments, antimetabolites suitable for use in the present compositions and methods include, but are not limited to: 1) folic acid analogs (*e.g.*, methotrexate (amethopterin)); 2) pyrimidine analogs (*e.g.*, fluorouracil (5-fluorouracil; 5-FU), floxuridine (fluorode-oxyuridine; FudR), and cytarabine (cytosine arabinoside)); and 3) purine analogs (*e.g.*, mercaptopurine (6-mercaptopurine; 6-MP), thioguanine (6-thioguanine; TG), and pentostatin (2'-deoxycofomycin)).

In still further embodiments, chemotherapeutic agents suitable for use in the compositions and methods of the present invention include, but are not limited to: 1) vinca alkaloids (*e.g.*, vinblastine (VLB), vincristine); 2) epipodophyllotoxins (*e.g.*, etoposide and teniposide); 3) antibiotics (*e.g.*, dactinomycin (actinomycin D), daunorubicin (daunomycin; 5 rubidomycin), doxorubicin, bleomycin, plicamycin (mithramycin), and mitomycin (mitomycin C)); 4) enzymes (*e.g.*, L-asparaginase); 5) biological response modifiers (*e.g.*, interferon- α); 6) platinum coordinating complexes (*e.g.*, cisplatin (cis-DDP) and carboplatin); 7) anthracenediones (*e.g.*, mitoxantrone); 8) substituted ureas (*e.g.*, hydroxyurea); 9) methylhydrazine derivatives (*e.g.*, procarbazine (N-methylhydrazine; 10 MIH)); 10) adrenocortical suppressants (*e.g.*, mitotane (o,p'-DDD) and aminoglutethimide); 11) adrenocorticosteroids (*e.g.*, prednisone); 12) progestins (*e.g.*, hydroxyprogesterone caproate, medroxyprogesterone acetate, and megestrol acetate); 13) estrogens (*e.g.*, diethylstilbestrol and ethinyl estradiol); 14) antiestrogens (*e.g.*, tamoxifen); 15) androgens (*e.g.*, testosterone propionate and fluoxymesterone); 16) antiandrogens (*e.g.*, flutamide); and 15 17) gonadotropin-releasing hormone analogs (*e.g.*, leuprolide).

Any oncolytic agent that is routinely used in a cancer therapy context finds use in the compositions and methods of the present invention. For example, the U.S. Food and Drug Administration maintains a formulary of oncolytic agents approved for use in the United States. International counterpart agencies to the U.S.F.D.A. maintain similar formularies. 20 Table 1 provides a list of exemplary antineoplastic agents approved for use in the U.S. Those skilled in the art will appreciate that the "product labels" required on all U.S. approved chemotherapeutics describe approved indications, dosing information, toxicity data, and the like, for the exemplary agents.

Table 1

Aldesleukin (des-alanyl-1, serine-125 human interleukin-2)	Proleukin	Chiron Corp., Emeryville, CA
Alemtuzumab (IgG1 κ anti CD52 antibody)	Campath	Millennium and ILEX Partners, LP, Cambridge, MA
Allopurinol (1,5-dihydro-4 H -pyrazolo[3,4-d]pyrimidin-4-one monosodium salt)	Zyloprim	GlaxoSmithKline, Research Triangle Park, NC
Altretamine (N,N,N',N',N'',N''-, hexamethyl-1,3,5-triazine-2,4, 6-triamine)	Hexalen	US Bioscience, West Conshohocken, PA
Amifostine	Ethyol	US Bioscience

(ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester))		
Anastrozole (1,3-Benzenediacetonitrile, a, a, a', a'-tetramethyl-5-(1H-1,2,4-triazol-1-ylmethyl))	Arimidex	AstraZeneca Pharmaceuticals, LP, Wilmington, DE
Arsenic trioxide	Trisenox	Cell Therapeutic, Inc., Seattle, WA
Asparaginase (L-asparagine amidohydrolase, type EC-2)	Elspar	Merck & Co., Inc., Whitehouse Station, NJ
BCG Live (lyophilized preparation of an attenuated strain of <i>Mycobacterium bovis</i> (<i>Bacillus Calmette-Guérin</i> [BCG], substrain Montreal)	TICE BCG	Organon Teknika, Corp., Durham, NC
bexarotene capsules (4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl) ethenyl] benzoic acid)	Targretin	Ligand Pharmaceuticals
bexarotene gel	Targretin	Ligand Pharmaceuticals
Bleomycin (cytotoxic glycopeptide antibiotics produced by <i>Streptomyces verticillus</i> ; bleomycin A ₂ and bleomycin B ₂)	Blenoxane	Bristol-Myers Squibb Co., NY, NY
Capecitabine (5'-deoxy-5-fluoro-N-[(pentylloxy)carbonyl]-cytidine)	Xeloda	Roche
Carboplatin (platinum, diammine [1,1-cyclobutanedicarboxylato(2-)-0, 0']-, (SP-4-2))	Paraplatin	Bristol-Myers Squibb
Carmustine (1,3-bis(2-chloroethyl)-1-nitrosourea)	BCNU, BiCNU	Bristol-Myers Squibb
Carmustine with Polifeprosan 20 Implant	Gliadel Wafer	Guilford Pharmaceuticals, Inc., Baltimore, MD
Celecoxib (as 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide)	Celebrex	Searle Pharmaceuticals, England
Chlorambucil (4-[bis(2chloroethyl)amino]benzenebutanoic acid)	Leukeran	GlaxoSmithKline
Cisplatin (PtCl ₂ H ₆ N ₂)	Platinol	Bristol-Myers Squibb
Cladribine (2-chloro-2'-deoxy-b-D-adenosine)	Leustatin, 2-CdA	R.W. Johnson Pharmaceutical Research Institute, Raritan, NJ
Cyclophosphamide (2-[bis(2-chloroethyl)amino] tetrahydro-2H-13,2-oxazaphosphorine 2-oxide monohydrate)	Cytosan, Neosar	Bristol-Myers Squibb
Cytarabine (1-b-D-Arabinofuranosylcytosine, C ₉ H ₁₃ N ₃ O ₅)	Cytosar-U	Pharmacia & Upjohn Company
cytarabine liposomal	DepoCyt	Skye Pharmaceuticals, Inc.,

		San Diego, CA
Dacarbazine (5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide (DTIC))	DTIC-Dome	Bayer AG, Leverkusen, Germany
Dactinomycin, actinomycin D (actinomycin produced by <i>Streptomyces parvullus</i> , C ₆₂ H ₈₆ N ₁₂ O ₁₆)	Cosmegen	Merck
Darbepoetin alfa (recombinant peptide)	Aranesp	Amgen, Inc., Thousand Oaks, CA
daunorubicin liposomal ((8S-cis)-8-acetyl-10-[(3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride)	DanuoXome	Nexstar Pharmaceuticals, Inc., Boulder, CO
Daunorubicin HCl, daunomycin ((1 S, 3 S)-3-Acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1-naphthacenyl 3-amino-2,3,6-trideoxy-(α)-L-lyxo-hexopyranoside hydrochloride)	Cerubidine	Wyeth Ayerst, Madison, NJ
Denileukin diftitox (recombinant peptide)	Ontak	Seragen, Inc., Hopkinton, MA
Dexrazoxane ((S)-4,4'-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione)	Zinecard	Pharmacia & Upjohn Company
Docetaxel ((2R,3S)-N-carboxy-3-phenylisoserine, N-tert-butyl ester, 13-ester with 5b-20-epoxy-12a,4,7b,10b,13a-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate)	Taxotere	Aventis Pharmaceuticals, Inc., Bridgewater, NJ
Doxorubicin HCl (8S,10S)-10-[(3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyl)oxy]-8-glycolyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacenedione hydrochloride)	Adriamycin, Rubex	Pharmacia & Upjohn Company
doxorubicin	Adriamycin PFS Intravenous injection	Pharmacia & Upjohn Company
doxorubicin liposomal	Doxil	Sequus Pharmaceuticals, Inc., Menlo park, CA
dromostanolone propionate (17 β -Hydroxy-2 α -methyl-5 α -androstan-3-one propionate)	Dromostanolone	Eli Lilly & Company, Indianapolis, IN
dromostanolone propionate	Masteron e injection	Syntex, Corp., Palo Alto, CA
Elliott's B Solution	Elliott's B Solution	Orphan Medical, Inc

Epirubicin ((8S-cis)-10-[(3-amino-2,3,6-trideoxy-a-L-arabino- hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione hydrochloride)	Ellence	Pharmacia & Upjohn Company
Epoetin alfa (recombinant peptide)	Epogen	Amgen, Inc
Estramustine (estra-1,3,5(10)-triene-3,17-diol(17(beta))-, 3-[bis(2-chloroethyl)carbamate] 17-(dihydrogen phosphate), disodium salt, monohydrate, or estradiol 3-[bis(2-chloroethyl)carbamate] 17-(dihydrogen phosphate), disodium salt, monohydrate)	Emcyt	Pharmacia & Upjohn Company
Etoposide phosphate (4'-Demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-(beta)-D-glucopyranoside], 4'-(dihydrogen phosphate))	Etoposide	Bristol-Myers Squibb
etoposide, VP-16 (4'-demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-(beta)-D-glucopyranoside])	Vepesid	Bristol-Myers Squibb
Exemestane (6-methylenandrosta-1,4-diene-3, 17-dione)	Aromasin	Pharmacia & Upjohn Company
Filgrastim (r-metHuG-CSF)	Neupogen	Amgen, Inc
floxuridine (intraarterial) (2'-deoxy-5-fluorouridine)	FUDR	Roche
Fludarabine (fluorinated nucleotide analog of the antiviral agent vidarabine, 9-b -D-arabinofuranosyladenine (ara-A))	Fludara	Berlex Laboratories, Inc., Cedar Knolls, NJ
Fluorouracil, 5-FU (5-fluoro-2,4(1H,3H)-pyrimidinedione)	Adrucil	ICN Pharmaceuticals, Inc., Humacao, Puerto Rico
Fulvestrant (7-alpha-[9-(4,4,5,5,5-penta fluoropentylsulphanyl) nonyl]estra-1,3,5-(10)-triene-3,17-beta-diol)	Faslodex	IPR Pharmaceuticals, Guayama, Puerto Rico
Gemcitabine (2'-deoxy-2', 2'-difluorocytidine monohydrochloride (b-isomer))	Gemzar	Eli Lilly
Gemtuzumab Ozogamicin (anti-CD33 hP67.6)	Mylotarg	Wyeth Ayerst
Goserelin acetate	Zoladex Implant	AstraZeneca Pharmaceuticals
Hydroxyurea	Hydrea	Bristol-Myers Squibb
Ibritumomab Tiuxetan (immunoconjugate resulting from a thiourea covalent bond between the monoclonal antibody	Zevalin	Biogen IDEC, Inc., Cambridge MA

Ibritumomab and the linker-chelator tiuxetan [N-[2-bis(carboxymethyl)amino]-3-(p- isothiocyanatophenyl)- propyl]-[N-[2- bis(carboxymethyl)amino]-2-(methyl) - ethyl]glycine)		
Idarubicin (5, 12-Naphthacenedione, 9-acetyl-7-[(3-amino- 2,3,6-trideoxy-(alpha)-L- lyxo - hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,9,11- trihydroxyhydrochloride, (7S- cis))	Idamycin	Pharmacia & Upjohn Company
Ifosfamide (3-(2-chloroethyl)-2-[(2- chloroethyl)amino]tetrahydro-2H-1,3,2- oxazaphosphorine 2-oxide)	IFEX	Bristol-Myers Squibb
Imatinib Mesilate (4-[(4-Methyl-1-piperaziny)methyl]-N-[4- methyl-3-[[4-(3-pyridinyl)-2- pyrimidinyl]amino]-phenyl]benzamide methanesulfonate)	Gleevec	Novartis AG, Basel, Switzerland
Interferon alfa-2a (recombinant peptide)	Roferon- A	Hoffmann-La Roche, Inc., Nutley, NJ
Interferon alfa-2b (recombinant peptide)	Intron A (Lyophilized Betaseron)	Schering AG, Berlin, Germany
Irinotecan HCl ((4S)-4,11-diethyl-4-hydroxy-9-[(4- piperi- dinopiperidino)carbonyloxy]-1H-pyrano[3', 4': 6,7] indolizino[1,2-b] quinoline-3,14(4H, 12H) dione hydrochloride trihydrate)	Camptosar	Pharmacia & Upjohn Company
Letrozole (4,4'-(1H-1,2,4 -Triazol-1-ylmethylene) dibenzonitrile)	Femara	Novartis
Leucovorin (L-Glutamic acid, N[4[[[(2-amino-5-formyl- 1,4,5,6,7,8 hexahydro-4-oxo-6- pteridinyl)methyl]amino]benzoyl], calcium salt (1:1))	Wellcovorin, Leucovorin	Immunex, Corp., Seattle, WA
Levamisole HCl ((-)-(S)-2,3,5, 6-tetrahydro-6-phenylimidazo [2,1-b] thiazole monohydrochloride C ₁₁ H ₁₂ N ₂ S·HCl)	Ergamisol	Janssen Research Foundation, Titusville, NJ
Lomustine (1-(2-chloro-ethyl)-3-cyclohexyl-1-nitrosourea)	CeeNU	Bristol-Myers Squibb
Mecllorethamine, nitrogen mustard (2-chloro-N-(2-chloroethyl)-N- methylethanamine hydrochloride)	Mustargen	Merck

Megestrol acetate 17 α (acetyloxy)- 6- methylpregna- 4,6- diene- 3,20- dione	Megace	Bristol-Myers Squibb
Melphalan, L-PAM (4-[bis(2-chloroethyl) amino]-L-phenylalanine)	Alkeran	GlaxoSmithKline
Mercaptopurine, 6-MP (1,7-dihydro-6 H -purine-6-thione monohydrate)	Purinethol	GlaxoSmithKline
Mesna (sodium 2-mercaptoethane sulfonate)	Mesnex	Asta Medica
Methotrexate (N-[4-[[[(2,4-diamino-6- pteridiny]methyl]methylamino]benzoyl]-L- glutamic acid)	Methotrex ate	Lederle Laboratories
Methoxsalen (9-methoxy-7H-furo[3,2-g][1]-benzopyran-7- one)	Uvadex	Therakos, Inc., Way Exton, Pa
Mitomycin C	Mutamycin	Bristol-Myers Squibb
mitomycin C	Mitozytre x	SuperGen, Inc., Dublin, CA
Mitotane (1,1-dichloro-2-(o-chlorophenyl)-2-(p- chlorophenyl) ethane)	Lysodren	Bristol-Myers Squibb
Mitoxantrone (1,4-dihydroxy-5,8-bis[[2- [(2- hydroxyethyl)amino]ethyl]amino]-9,10- anthracenedione dihydrochloride)	Novantro ne	Immunex Corporation
Nandrolone phenpropionate	Durabolin -50	Organon, Inc., West Orange, NJ
Nofetumomab	Verluma	Boehringer Ingelheim Pharma KG, Germany
Oprelvekin (IL-11)	Neumega	Genetics Institute, Inc., Alexandria, VA
Oxaliplatin (cis-[(1R,2R)-1,2-cyclohexanediamine-N,N'] [oxalato(2-)-O,O'] platinum)	Eloxatin	Sanofi Synthelabo, Inc., NY, NY
Paclitaxel (5 β , 20-Epoxy-1,2a, 4,7 β , 10 β , 13a- hexahydroxytax-11-en-9-one 4,10-diacetate 2- benzoate 13-ester with (2R, 3 S)- N-benzoyl-3- phenylisoserine)	TAXOL	Bristol-Myers Squibb
Pamidronate (phosphonic acid (3-amino-1- hydroxypropylidene) bis-, disodium salt, pentahydrate, (APD))	Aredia	Novartis
Pegademase ((monomethoxypolyethylene glycol succinimidyl) 11 - 17 -adenosine deaminase)	Adagen (Pegadem ase	Enzon Pharmaceuticals, Inc., Bridgewater, NJ

	Bovine)	
Pegaspargase (monomethoxypolyethylene glycol succinimidyl L-asparaginase)	Oncaspar	Enzon
Pegfilgrastim (covalent conjugate of recombinant methionyl human G-CSF (Filgrastim) and monomethoxypolyethylene glycol)	Neulasta	Amgen, Inc
Pentostatin	Nipent	Parke-Davis Pharmaceutical Co., Rockville, MD
Pipobroman	Vercyte	Abbott Laboratories, Abbott Park, IL
Plicamycin, Mithramycin (antibiotic produced by <i>Streptomyces plicatus</i>)	Mithracin	Pfizer, Inc., NY, NY
Porfimer sodium	Photofrin	QLT Phototherapeutics, Inc., Vancouver, Canada
Procarbazine (N-isopropyl-μ-(2-methylhydrazino)-p- toluamide monohydrochloride)	Matulane	Sigma Tau Pharmaceuticals, Inc., Gaithersburg, MD
Quinacrine (6-chloro-9-(1-methyl-4-diethyl-amine) butylamino-2-methoxyacridine)	Atabrine	Abbott Labs
Rasburicase (recombinant peptide)	Elitek	Sanofi-Synthelabo, Inc.,
Rituximab (recombinant anti-CD20 antibody)	Rituxan	Genentech, Inc., South San Francisco, CA
Sargramostim (recombinant peptide)	Prokine	Immunex Corp
Streptozocin (streptozocin 2-deoxy-2- [[[(methylnitrosoamino)carbonyl]amino]-a-(and b)-D-glucopyranose and 220 mg citric acid anhydrous)	Zanosar	Pharmacia & Upjohn Company
Talc (Mg ₃ Si ₄ O ₁₀ (OH) ₂)	Sclerosol	Bryan, Corp., Woburn, MA
Tamoxifen ([(Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N, N-dimethylethanamine 2-hydroxy-1,2,3- propanetricarboxylate (1:1)])	Nolvadex	AstraZeneca Pharmaceuticals
Temozolomide (3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-as- tetrazine-8-carboxamide)	Temodar	Schering
teniposide, VM-26 (4'-demethylepipodophyllotoxin 9-[4,6-O-(R)-2- thenylidene-(beta)-D-glucopyranoside])	Vumon	Bristol-Myers Squibb
Testolactone (13-hydroxy-3-oxo-13,17-secoandrosta-1,4- dien-17-oic acid [dgr]-lactone)	Teslac	Bristol-Myers Squibb

Thioguanine, 6-TG (2-amino-1,7-dihydro-6 H - purine-6-thione)	Thioguanine	GlaxoSmithKline
Thiotepa (Aziridine, 1,1',1''-phosphinothioylidynetris-, or Tris (1-aziridiny) phosphine sulfide)	Thioplex	Immunex Corporation
Topotecan HCl ((S)-10-[(dimethylamino) methyl]-4-ethyl-4,9-dihydroxy-1H-pyrano[3', 4': 6,7] indolizino [1,2-b] quinoline-3,14-(4H,12H)-dione monohydrochloride)	Hycamtin	GlaxoSmithKline
Toremifene (2-(p-[(Z)-4-chloro-1,2-diphenyl-1-butenyl]-phenoxy)-N,N-dimethylethylamine citrate (1:1))	Fareston	Roberts Pharmaceutical Corp., Eatontown, NJ
Tositumomab, I 131 Tositumomab (recombinant murine immunotherapeutic monoclonal IgG _{2a} lambda anti-CD20 antibody (I 131 is a radioimmunotherapeutic antibody))	Bexxar	Corixa Corp., Seattle, WA
Trastuzumab (recombinant monoclonal IgG ₁ kappa anti-HER2 antibody)	Herceptin	Genentech, Inc
Uracil Mustard	Uracil Mustard Capsules	Roberts Labs
Valrubicin, N-trifluoroacetyl Adriamycin-14-valerate ((2S-cis)-2- [1,2,3,4,6,11-hexahydro-2,5,12-trihydroxy-7 methoxy-6,11-dioxo-[[4 2,3,6-trideoxy-3- [(trifluoroacetyl)-amino- α -L-lyxo-hexopyranosyl]oxyl]-2-naphthacenyl]-2-oxoethyl pentanoate)	Valstar	Anthra --> Medeva
Vinblastine, Leurocristine (C ₄₆ H ₅₆ N ₄ O ₁₀ •H ₂ SO ₄)	Velban	Eli Lilly
Vincristine (C ₄₆ H ₅₆ N ₄ O ₁₀ •H ₂ SO ₄)	Oncovin	Eli Lilly
Vinorelbine (3',4'-didehydro-4'-deoxy-C'-norvincal leukoblastine [R-(R*,R*)-2,3-dihydroxybutanedioate (1:2)(salt)])	Navelbine	GlaxoSmithKline
Zoledronate, Zoledronic acid ((1-Hydroxy-2-imidazol-1-yl-phosphonoethyl) phosphonic acid monohydrate)	Zometa	Novartis

Anticancer agents further include compounds which have been identified to have anticancer activity. Examples include, but are not limited to, 3-AP, 12-O-tetradecanoylphorbol-13-acetate, 17AAG, 852A, ABI-007, ABR-217620, ABT-751, ADI-PEG 20, AE-941, AG-013736, AGRO100, alanosine, AMG 706, antibody G250,

antineoplastons, AP23573, apaziquone, APC8015, atiprimod, ATN-161, atrasenten, azacitidine, BB-10901, BCX-1777, bevacizumab, BG00001, bicalutamide, BMS 247550, bortezomib, bryostatin-1, buserelin, calcitriol, CCI-779, CDB-2914, cefixime, cetuximab, CG0070, cilengitide, clofarabine, combretastatin A4 phosphate, CP-675,206, CP-724,714, CpG 7909, curcumin, decitabine, DENSPM, doxercalciferol, E7070, E7389, ecteinascidin 743, efaproxiral, eflornithine, EKB-569, enzastaurin, erlotinib, exisulind, flavopiridol, fludarabine, flutamide, fotemustine, FR901228, G17DT, galiximab, gefitinib, genistein, glufosfamide, GTI-2040, histrelin, HKI-272, homoharringtonine, HSPPC-96, hu14.18-interleukin-2 fusion protein, HuMax-CD4, iloprost, imiquimod, infliximab, interleukin-12, IPI-504, irofulven, ixabepilone, lapatinib, lenalidomide, lestaurtinib, leuprolide, LMB-9 immunotoxin, lonafarnib, luniliximab, mafosfamide, MB07133, MDX-010, MLN2704, monoclonal antibody 3F8, monoclonal antibody J591, motexafin, MS-275, MVA-MUC1-IL2, nilutamide, nitrocamptothecin, nolatrexed dihydrochloride, nolvadex, NS-9, O6-benzylguanine, oblimersen sodium, ONYX-015, oregovomab, OSI-774, panitumumab, paraplatin, PD-0325901, pemetrexed, PHY906, pioglitazone, pirfenidone, pixantrone, PS-341, PSC 833, PXD101, pyrazoloacridine, R115777, RAD001, ranpirnase, rebeccamycin analogue, rhuAngiostatin protein, rhuMab 2C4, rosiglitazone, rubitecan, S-1, S-8184, satraplatin, SB-, 15992, SGN-0010, SGN-40, sorafenib, SR31747A, ST1571, SU011248, suberoylanilide hydroxamic acid, suramin, talabostat, talampanel, tariquidar, temsirolimus, TGFa-PE38 immunotoxin, thalidomide, thymalfasin, tipifarnib, tirapazamine, TLK286, trabectedin, trimetrexate glucuronate, TroVax, UCN-1, valproic acid, vinflunine, VNP40101M, volociximab, vorinostat, VX-680, ZD1839, ZD6474, zileuton, and zosuquidar trihydrochloride.

For a more detailed description of anticancer agents and other therapeutic agents, those skilled in the art are referred to any number of instructive manuals including, but not limited to, the Physician's Desk Reference and to Goodman and Gilman's "Pharmaceutical Basis of Therapeutics" tenth edition, Eds. Hardman *et al.*, 2002.

The present invention provides methods for administering a compound of the invention with radiation therapy. The invention is not limited by the types, amounts, or delivery and administration systems used to deliver the therapeutic dose of radiation to an animal. For example, the animal may receive photon radiotherapy, particle beam radiation therapy, other types of radiotherapies, and combinations thereof. In some embodiments, the radiation is delivered to the animal using a linear accelerator. In still other embodiments, the radiation is delivered using a gamma knife.

The source of radiation can be external or internal to the animal. External radiation therapy is most common and involves directing a beam of high-energy radiation to a tumor site through the skin using, for instance, a linear accelerator. While the beam of radiation is localized to the tumor site, it is nearly impossible to avoid exposure of normal, healthy tissue.

5 However, external radiation is usually well tolerated by animals. Internal radiation therapy involves implanting a radiation-emitting source, such as beads, wires, pellets, capsules, particles, and the like, inside the body at or near the tumor site including the use of delivery systems that specifically target cancer cells (*e.g.*, using particles attached to cancer cell binding ligands). Such implants can be removed following treatment, or left in the body
10 inactive. Types of internal radiation therapy include, but are not limited to, brachytherapy, interstitial irradiation, intracavity irradiation, radioimmunotherapy, and the like.

The animal may optionally receive radiosensitizers (*e.g.*, metronidazole, misonidazole, intra-arterial Budr, intravenous iododeoxyuridine (IudR), nitroimidazole, 5-substituted-4-nitroimidazoles, 2H-isoindolediones, [[(2-bromoethyl)-amino]methyl]-nitro-
15 1H-imidazole-1-ethanol, nitroaniline derivatives, DNA-affinic hypoxia selective cytotoxins, halogenated DNA ligand, 1,2,4 benzotriazine oxides, 2-nitroimidazole derivatives, fluorine-containing nitroazole derivatives, benzamide, nicotinamide, acridine-intercalator, 5-thiotetrazole derivative, 3-nitro-1,2,4-triazole, 4,5-dinitroimidazole derivative, hydroxylated texaphrins, cisplatin, mitomycin, tiripazamine, nitrosourea, mercaptopurine, methotrexate,
20 fluorouracil, bleomycin, vincristine, carboplatin, epirubicin, doxorubicin, cyclophosphamide, vindesine, etoposide, paclitaxel, heat (hyperthermia), and the like), radioprotectors (*e.g.*, cysteamine, aminoalkyl dihydrogen phosphorothioates, amifostine (WR 2721), IL-1, IL-6, and the like). Radiosensitizers enhance the killing of tumor cells. Radioprotectors protect healthy tissue from the harmful effects of radiation.

25 Any type of radiation can be administered to an animal, so long as the dose of radiation is tolerated by the animal without unacceptable negative side-effects. Suitable types of radiotherapy include, for example, ionizing (electromagnetic) radiotherapy (*e.g.*, X-rays or gamma rays) or particle beam radiation therapy (*e.g.*, high linear energy radiation). Ionizing radiation is defined as radiation comprising particles or photons that have sufficient energy to
30 produce ionization, *i.e.*, gain or loss of electrons (as described in, for example, U.S. 5,770,581 incorporated herein by reference in its entirety). The effects of radiation can be at least partially controlled by the clinician. In one embodiment, the dose of radiation is fractionated for maximal target cell exposure and reduced toxicity.

In one embodiment, the total dose of radiation administered to an animal is about .01 Gray (Gy) to about 100 Gy. In another embodiment, about 10 Gy to about 65 Gy (*e.g.*, about 15 Gy, 20 Gy, 25 Gy, 30 Gy, 35 Gy, 40 Gy, 45 Gy, 50 Gy, 55 Gy, or 60 Gy) are administered over the course of treatment. While in some embodiments a complete dose of radiation can be administered over the course of one day, the total dose is ideally fractionated and administered over several days. Desirably, radiotherapy is administered over the course of at least about 3 days, *e.g.*, at least 5, 7, 10, 14, 17, 21, 25, 28, 32, 35, 38, 42, 46, 52, or 56 days (about 1-8 weeks). Accordingly, a daily dose of radiation will comprise approximately 1-5 Gy (*e.g.*, about 1 Gy, 1.5 Gy, 1.8 Gy, 2 Gy, 2.5 Gy, 2.8 Gy, 3 Gy, 3.2 Gy, 3.5 Gy, 3.8 Gy, 4 Gy, 4.2 Gy, or 4.5 Gy), or 1-2 Gy (*e.g.*, 1.5-2 Gy). The daily dose of radiation should be sufficient to induce destruction of the targeted cells. If stretched over a period, in one embodiment, radiation is not administered every day, thereby allowing the animal to rest and the effects of the therapy to be realized. For example, radiation desirably is administered on 5 consecutive days, and not administered on 2 days, for each week of treatment, thereby allowing 2 days of rest per week. However, radiation can be administered 1 day/week, 2 days/week, 3 days/week, 4 days/week, 5 days/week, 6 days/week, or all 7 days/week, depending on the animal's responsiveness and any potential side effects. Radiation therapy can be initiated at any time in the therapeutic period. In one embodiment, radiation is initiated in week 1 or week 2, and is administered for the remaining duration of the therapeutic period. For example, radiation is administered in weeks 1-6 or in weeks 2-6 of a therapeutic period comprising 6 weeks for treating, for instance, a solid tumor. Alternatively, radiation is administered in weeks 1-5 or weeks 2-5 of a therapeutic period comprising 5 weeks. These exemplary radiotherapy administration schedules are not intended, however, to limit the present invention.

In some embodiments of the present invention, an inhibitor of myeloid leukemia cell growth (*e.g.*, MDI 301) and one or more therapeutic agents or anticancer agents are administered to an animal under one or more of the following conditions: at different periodicities, at different durations, at different concentrations, by different administration routes, *etc.* In some embodiments, the compound is administered prior to the therapeutic or anticancer agent, *e.g.*, 0.5, 1, 2, 3, 4, 5, 10, 12, or 18 hours, 1, 2, 3, 4, 5, or 6 days, or 1, 2, 3, or 4 weeks prior to the administration of the therapeutic or anticancer agent. In some embodiments, the inhibitor of myeloid leukemia cell growth (*e.g.*, MDI 301) is administered after the therapeutic or anticancer agent, *e.g.*, 0.5, 1, 2, 3, 4, 5, 10, 12, or 18 hours, 1, 2, 3, 4, 5, or 6 days, or 1, 2, 3, or 4 weeks after the administration of the anticancer agent. In some

embodiments, the inhibitor of myeloid leukemia cell growth (e.g., MDI 301) and the therapeutic or anticancer agent are administered concurrently but on different schedules, *e.g.*, the inhibitor of myeloid leukemia cell growth (e.g., MDI 301) is administered daily while the therapeutic or anticancer agent is administered once a week, once every two weeks, once every three weeks, or once every four weeks. In other embodiments, the inhibitor of myeloid leukemia cell growth (e.g., MDI 301) is administered once a week while the therapeutic or anticancer agent is administered daily, once a week, once every two weeks, once every three weeks, or once every four weeks.

One of ordinary skill in the art will readily recognize that the foregoing represents merely a detailed description of certain preferred embodiments of the present invention. Various modifications and alterations of the compositions and methods described above can readily be achieved using expertise available in the art and are within the scope of the invention.

EXAMPLES

The invention, now being generally described, will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

Example 1

This example provides the materials and methods for Examples 2-7.

MDI 301, ATRA and 9-cis retinoic acid. MDI 301 is a 9-cis retinoic acid derivative in which the terminal carboxylic acid group has been replaced by a picolinate ester (see, e.g., U.S. Patent No. 5,837,728; Varani J, et al., Arch. Dermatol. Res. 2003;295(6):255-62). MDI 301 is an agonist for retinoic acid receptor- α (RAR α) (activation >100X at 1 nM) and RAR γ (100X at 1 nM). MDI 301 also demonstrates weak activity for RAR α as well as weak activity for retinoic acid receptor X- α (RXR α) and RXR β while 9-cis retinoic acid is a high affinity ligand for the RXR and binds to RAR (see, e.g., Kizaki, et al., Blood 1993 82(12)-3592-3599). The retinoid was dissolved in dimethyl sulfoxide (DMSO) at a 1.0% concentration and frozen at -80°C protected from light. ATRA and 9-cis retinoic acid were purchased from Sigma Chemical Company (St. Louis, MO) and handled exactly as MDI 301.

Tumor cell lines. Three human acute myeloid leukemia (AML) cell lines – i.e., HL60, NB4, and OCI-M2 - and a human chronic myeloid leukemia (CML) cell line – i.e., K562 - were used. All were obtained from ATCC. The K562 and OCI-M2 myeloid / erythroid leukemia cell lines were derived from a patient with a blast phase of chronic myelogenous leukemia and a patient with high-grade myelodysplastic syndrome, respectively. NB4 was originally derived from a patient with acute promyelocytic leukemia (PML) (AML-M3 in FAB classification) with the classical t(15;17) translocation. The HL60 line, though often referred to as an AML-M3, likely reflects a more primitive variant (i.e., AML-M2). Both NB4 and HL60 cells are able to differentiate into more mature granulocytes in response to ATRA, even though HL60 cells do not have t(15;17) and are less responsive to ATRA than NB4 (see, e.g., Barber N, et al., *Leuk Res.* 2008;32(2):315-22; Breitman TR, et al., *Proc Natl Acad Sci USA.* 1980;77(5):2936-40). In contrast to the effects of ATRA, HL60 cells differentiate into monocytic, macrophage-like cells in response to 1,25-dihydroxyvitamin (D₃), 12-O-tetradecanoylphorbol-13-acetate (TPA) and GM-CSF (see, e.g., Ostrem VK, et al., *J Biol Chem.* 1987;262(29):14164-71; Ryves WJ, et al., *Carcinogenesis.* 1994;15(11):2501-6). HL60, NB4 and K562 cells were grown in RPMI-1640 medium with 10% fetal bovine serum (FBS). OCI-M2 cells were grown in Iscove's MEM containing 20% heat-inactivated FBS. Growth was at 37°C in an atmosphere of 95% air and 5% CO₂. Each of these cell types grow in suspension, and were "passaged" on a 3-4 day cycle.

In vitro treatment protocols and endpoints. Concentration-dependent and time-dependent growth inhibition studies were carried out *in vitro*. Cells were plated in wells of a 24-well dish at 5×10^4 cells per well in their respective growth media. MDI 301 and ATRA were added at the desired concentrations and the cells incubated for 1, 2 or 3 days. At the end of the incubation period, cells were counted using an electronic particle counter. Viability was determined by exclusion of trypan blue.

To assess adhesion, cells were treated for three days with either of the two retinoids (or control) in growth medium. At the end of the incubation period, the non-attached cells were harvested and the plate containing the attached cells gently washed 1X and then treated with 10% buffered formalin. Attached cells were enumerated and wells were photographed under phase-contrast microscopy.

In parallel, cells were assessed by flow cytometry for expression of CD11b and CD18. Two direct-conjugated primary antibodies (PE-labeled mouse anti-human CD18 and

APC-labeled mouse anti-human CD11b) were obtained from BD Biosciences (San Jose, CA). PE-labeled and APC-labeled IgGs were used as controls. Flow cytometric analysis was done using a BD LSRII cytometer, and data were analyzed using Verity Winlist 3D software. Briefly, cells in suspension were concentrated to $3\text{--}5 \times 10^6/\text{mL}$, washed 2X in cold Standard Flow Buffer (SFB) and pelleted. Cells were then resuspended at 5×10^5 in 100 μL of cold SFB in wells of a 96-well plate. The antibodies were added to the cells (1 $\mu\text{g}/100 \mu\text{L}$) and incubated for 30 minutes in the cold. After two wash steps, the cells were fixed in 250 μL of 2% formaldehyde solution per well. Cells were then assessed immediately or stored in the dark for up to 24 hours.

***In vivo* tumor growth inhibition.** Capacity to inhibit HL60 tumor cell growth in athymic mice was used to compare *in vivo* activity of the two retinoids. Athymic nude mice (Charles River, Wilmington, MA) were inoculated in the hind limb with 1×10^7 HL60 cells. The number of cells was chosen, based on preliminary studies showing that this dose of cells would produce tumors in the majority of the mice. Groups included control mice (injected with tumor cells but not retinoid-treated) and tumor-injected mice treated with either ATRA or MDI 301 at 20 mg/kg. Treatment was initiated at day 3 after tumor cell injection and consisted of up to 12 injections three days apart. In addition to the tumor-bearing animals, three mice that were not injected with tumor cells received the same retinoid treatment on the same schedule as the tumor-injected animals.

Tumor volume measurements were made throughout the course of the experiments. Specifically, cross-sectional diameter of the tumor in three planes was used to estimate tumor size. Tumor-bearing mice were sacrificed at day 33, or sooner if the tumor size reached a volume that impeded normal behavior or if the tumor demonstrated signs of incipient ulceration (i.e., if it became red and inflamed). At the time of sacrifice, tumor tissue along with surrounding normal tissue was removed and fixed in 10% buffered formalin. Formalin-fixed tissue was paraffin-embedded, stained with hematoxylin and eosin and examined at the light microscopic level.

During the retinoid-treatment phase, mice were examined daily for signs of skin irritations. Three parameters – i) redness / edema, ii) dryness / cracking and iii) flaking - were scored on a 0 – 4+ scale and the combined score of the three parameters was used as a measure of skin irritation. Each animal was also weighed at weekly intervals.

Assessment of pro-inflammatory cytokines. Approximately 400 μ L of blood was obtained from each animal by heart stick at the time of sacrifice. A panel of pro-inflammatory cytokines, including tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), keratinocyte-derived chemokine (KC; analogue of human IL-8) and macrophage chemotactic peptide-1 (MCP-1) was assessed. Levels of each were determined using the Bio-Plex bead-based cytokine assay from Bio-Rad Laboratories (Hercules, CA).

Statistical analysis. For *in vitro* proliferation studies, cells were examined in two or more separate experiments with duplicate or triplicate samples per data point. The percent inhibition was calculated for each retinoid concentration. Results from multiple experiments were then combined to obtain means and standard deviations for percent inhibition values. *In vivo* growth data (tumor volumes) were collected and plotted for individual mice over time. Average tumor volumes at the time of euthanasia for animals in each treatment group were determined. Skin irritation measurements were obtained daily and cytokine levels were assessed in serum taken at the time of euthanasia. Group means were compared by ANOVA followed by paired-group comparisons. Differences from control at $P < 0.05$ were considered significant.

Example 2.

This example describes a comparison of ATRA and MDI 301 for inhibition of tumor cell proliferation *in vitro*. MDI 301 has shown potential as a non-irritating skin-repair agent (see, e.g., Varani J, et al., Arch Dermatol Res. 2007;298(9):439-48; Varani J, et al., Arch. Dermatol. Res. 2003;295(6):255-62; Warner RL, et al., Wound Repair Regen. 2008;16(1):117-24; Dame MK, et al., In Vitro Cell Dev Biol Anim. 2009;45(9):551-7; Zeng W, et al., J Diabetes Complications. 2011;25(6):398-404), but its capacity to reduce growth and foster differentiation in myeloid leukemia cells has not yet been established. In order to determine how the experimental retinoid might compare with ATRA as an anti-cancer agent for this disease, a series of *in vitro* studies were carried out in which four different myeloid leukemia cell lines were treated with ATRA, MDI 301 and 9-cis RA over a range of concentrations. With all four cell lines, growth inhibition was observed with both MDI 301 and ATRA while 9-cis RA was less inhibitory. In all cases, MDI 301 was equivalent to (or slightly better than) ATRA (Fig. 1A). Time-dependent studies showed growth inhibition with HL60 cells as early as day-1. By day-3, differences between control HL60 cells and

cells exposed to either retinoid were statistically significant (Fig. 1B). A similar time-dependent inhibition was observed with the other cell lines as well.

Example 3.

5 This example describes induction of cell-substrate adhesion: comparison of ATRA and MDI 301. Induction of differentiation in myeloid leukemia cells is associated with an increase in cell-substrate adhesion. Fig. 2 compares ATRA and MDI 301 for ability to stimulate cell-substrate attachment in NB4, HL60 and K562 cells. A comparable increase in attachment of NB4 and HL60 cells was observed with the two agents. As can also be seen in 10 the Fig. 2, a change in morphology from round to flattened was clearly visible in some of the retinoid-treated NB4 cells. A similar change in HL60 morphology also occurred. K562 cells were loosely adherent in the absence of retinoid treatment and the increase with either agent was small.

15 Example 4.

This example describes CD11b and CD18 expression: comparison of ATRA and MDI 301. Table 2 summarizes effects of the two retinoids on expression of CD11b and CD18 in the myeloid leukemia lines. CD11b was up-regulated by ATRA and MDI 301 in NB4 and HL60 cells but there was little change in expression in K562 cells. In contrast, 20 CD18 was expressed on NB4 and HL60 cells in the absence of treatment and there was little change following exposure to either retinoid.

Table 2. CD11b and CD18 expression in myeloid leukemia cells: Effects on ATRA and MDI 301 on expression.

25	Cell line	% positive (mcf) CD11b		% positive (mcf) CD18	
30	NB4				
	Control	1	141	98	86
	ATRA	53	949 (6.7)	99	78
	(0.91)				
	MDI 301	76	1374 (9.7)	94	80
35	(0.93)				
	HL60				
	Control	5	81	98	85
	ATRA	36	141 (1.7)	95	60
	(0.70)				
40	MDI 301	38	410 (5.1)	94	75
	(0.88)				

K562			
Control	1	156	Not done
ATRA	2	197 (1.3)	Not done
MDI 301	4	288 (1.8)	Not done

The initial fluorescence gates were based on control cells incubated with PE-labeled IgG and APC-labeled IgG for CD11b and CD18, respectively. Control cells and retinoid treated cells were incubated for three days and then treated with the appropriate antibodies. Following this, samples were analyzed by flow cytometry as described in the Materials and Methods Section.

The value for % positive cells in each case represents the percentage of cells that are beyond the gate set with control cells. Values shown are for the specific antibodies. The percentage of positive cells were in the 2-3% positive range.

Mean fluorescence channel (mfc) values are presented as channel number and the number in parenthesis is a fold-change in the retinoid-treated cells as compared to control.

Values are from a single experiment, representative of three experiments.

Example 5.

This example provides a comparison of ATRA and MDI 301 for inhibition of HL60 growth *in vivo*. Fig. 3 shows tumor growth over time in individual control mice and individual retinoid-treated mice. For these studies, 1×10^7 HL60 cells were injected subcutaneously on day-zero. Control mice received intraperitoneal saline injection 3X per week beginning on day-3, while retinoid-treatment consisted of intraperitoneal injection of ATRA or MDI 301 at a concentration of 20 mg/kg over the same time-schedule. Consistent with what has been reported previously concerning HL60 growth in athymic nude mice (see, e.g., Dias S, et al., Proc Natl Acad Sci USA. 2001;98(19):10857-62), tumors in individual animals developed at widely varying rates. In some animals, evidence of tumor growth was apparent as early as day-6 while in other animals, tumor growth was not evident until between days 10 and 20. If tumor growth was not seen by day-20, it was not observed subsequently till the termination point (up to day-33). Overall, the average tumor volumes at the time of euthanasia were $289 \pm 157 \text{ mm}^3$ for control mice (n=5); $178 \pm 82 \text{ mm}^3$ in ATRA-treated mice (n=6) and $122 \pm 71 \text{ mm}^3$ in mice treated with MDI 301 (n=5) ($P < 0.05$ for tumors in MDI 301-treated mice versus control mice).

As can be seen from the Fig. 3 insert, there was little difference in gross tumor appearance (other than in size) to distinguish the treatment groups. Likewise, examination of hematoxylin and eosin – stained tissue sections revealed no significant histological

differences. Tumors in all three treatment groups were characterized as sheets of undifferentiated cells with areas of necrosis within the tumor parenchyma. Invasive borders could be seen in tumors of all three groups. However, a complete necropsy revealed no distant metastases in any organ.

5

Example 6.

This examples describes the toxicity in mice treated with ATRA and MDI 301. Skin irritation, characterized by redness / edema, dryness / cracking and flaking, is a common consequence of topical retinoid use (see, e.g., Standeven AM, et al., *Toxicol Appl Pharmacol.* 10 1996;138(1):169-75; Kim BH, et al., *Toxicol Lett.* 2003;146(1):65-73). The same parameters in the skin of athymic mice treated systemically with either ATRA or MDI 301 were assessed. A prominent response was seen in the ATRA-treated mice (Fig. 4). The response could be seen in some animals after as little as 3 days of treatment. As is typical with retinoid irritation, symptoms waxed and waned over time. In contrast to ATRA, animals treated with 15 MDI 301 showed little evidence of skin irritation at any time during the treatment phase (Fig. 4).

Weight loss is also commonly observed in mice treated topically with ATRA (see, e.g., Varani J, Fligiel H, Zhang J, Aslam MN, et al. Separation of retinoid-induced epidermal and dermal thickening from skin irritation. *Arch. Dermatol. Res.* 2003;295(6):255-62). 20 Consistent with this, mice treated with ATRA lost an average of 13% of their starting weight over the course of the treatment period while mice treated with MDI 301 lost 4%. Control mice, in contrast to mice treated with either retinoid, gained weight (7% average) over the period of observation.

25 **Example 7.**

This example describes the generation of pro-inflammatory mediators: a comparison of ATRA and MDI 301. Retinoid-induced skin irritation and weight loss are thought to reflect the generation of an inflammatory response. As a way to establish that this was, in fact, occurring, a number of different pro-inflammatory cytokines were assessed in serum 30 obtained at euthanasia. The data shown in Fig. 5 demonstrate that ATRA treatment increased the levels of several pro-inflammatory cytokines as compared to control, while MDI 301 did not. It can be seen from the figure that individual animals varied greatly in cytokine levels. While cytokine up-regulation was clearly associated with ATRA exposure, there did not appear to be a relationship between cytokine levels and tumor burden. In fact, along with

tumor-injected animals, three animals per group were exposed to ATRA and MDI 301 over the same treatment phase but not tumor cell-injected. Cytokine induction by ATRA occurred in these animals as well. For example, the IL-6 level was 24 ± 10 pg/mL in ATRA-treated mice versus 5 ± 5 pg/mL in control mice ($p < 0.05$). With KC, the level was 58 ± 27 pg/mL versus 15 ± 9 pg/mL ($p < 0.05$) and with MCP-1, the level was 50 ± 32 pg/mL versus 40 ± 34 pg/mL. In contrast, the non-tumor-injected animals exposed to MDI 301, like those that were tumor-injected, did not generate a cytokine response (Levels of IL-6, KC and MCP-1 were 3 ± 1 pg/mL, 10 ± 3 pg/mL and 32 ± 34 pg/mL, respectively).

10 INCORPORATION BY REFERENCE

The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

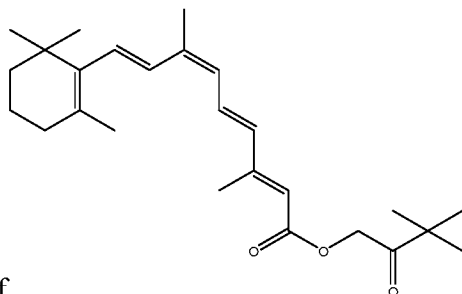
EQUIVALENTS

15 The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the
20 claims are intended to be embraced therein.

We claim:

1. A method of treating, ameliorating, or preventing recurrence of a condition involving myeloid leukemia cell growth in a patient comprising administering to said patient a
 5 therapeutically effective amount of a *cis*-derivative of retinoic acid, including salts, esters and prodrugs thereof, and a pharmaceutically acceptable carrier.

2. The method of claim 1, wherein the *cis*-derivative of retinoic acid is selected from the



group consisting of

- 10 (MDI 301), 1-(9-cis-retinoyloxy)-2-propanone, 1-(9-cis-retinoyloxy)-3-decanoyloxy-2-propanone, 1,3-bis-(9-cis-retinoyloxy)-2-propanone, 1-(9-cis-retinoyloxy)-2-pinacolone, 2-(9-cis-retinoyloxy)-acetophenone, 9-cis-retinoyloxy methyl 2,2-dimethyl propanoate, 2-(9-cis-retinoyloxy)-n-methyl-acetamide, 1-(9-cis-retinoyloxy)-3-hydroxy-2-propanone, 1-(9-cis-retinoyloxy)-2,3-dioleoylpropanone, and succinimidyl 9-cis-retinoate.

15

3. The method of claim 1, wherein the administering does not induce a cytokine response, an inflammatory response, and/or systemic toxicity in the patient.

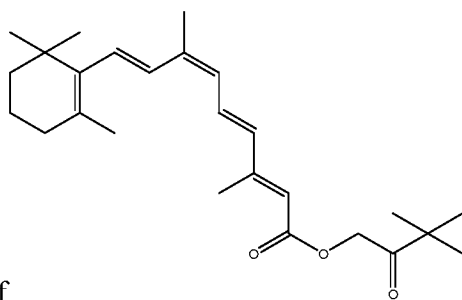
4. The method of claim 1, wherein the administering does not induce retinoic acid
 20 syndrome in the patient.

5. The method of claim 1, wherein the condition involving myeloid leukemia cell growth is one or more conditions selected from AML, PML, CML, and myelodysplastic anemia.

25

6. The method of claim 1, wherein the condition involving myeloid leukemia cell growth is PML.

7. The method of claim 1, wherein said patient is a human patient.
8. The method of claim 1, further comprising administering to said patient one or more anticancer agents.
9. The method of claim 8, wherein said anticancer agent is a chemotherapeutic agent.
10. The method of claim 8, wherein said anticancer agent is radiation therapy.
11. A kit comprising a pharmaceutical composition comprising a *cis*-derivative of retinoic acid, and instructions for administering said pharmaceutical composition to a patient diagnosed with AML and/or PML.
12. The kit of claim 11, further comprising one or more anticancer agents.
13. The kit of claim 11, wherein said pharmaceutical composition is to be administered together with one or more anticancer agents.
14. The kit of claim 11, wherein the pharmaceutical composition comprising a *cis*-derivative of retinoic acid does not induce a cytokine response, an inflammatory response, and/or systemic toxicity in the patient.
15. The kit of claim 11, wherein the pharmaceutical composition comprising a *cis*-derivative of retinoic acid does not induce retinoic acid syndrome in the patient.
16. The kit of claim 11, wherein the *cis*-derivative of retinoic acid is selected from the



group consisting of (MDI 301), 1-(9-cis-retinoyloxy)-2-propanone, 1-(9-cis-retinoyloxy)-3-decanoyloxy-2-propanone, 1,3-bis-(9-cis-retinoyloxy)-2-

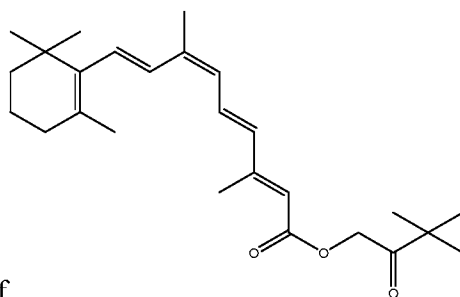
propanone, 1-(9-cis-retinoyloxy)-2-pinacolone, 2-(9-cis-retinoyloxy)-acetophenone, 9-cis-retinoyloxy methyl 2,2-dimethyl propanoate, 2-(9-cis-retinoyloxy)-n-methyl-acetamide, 1-(9-cis-retinoyloxy)-3-hydroxy-2-propanone, 1-(9-cis-retinoyloxy)-2,3-dioleoylpropanone, and succinimidyl 9-cis-retinoate.

5

17. A method of inhibiting myeloid leukemia cell growth, comprising exposing to a sample comprising proliferating myeloid leukemia cells a composition comprising a *cis*-derivative of retinoic acid, wherein the exposing results in inhibition of myeloid leukemia cell growth.

10

18. The method of claim 17, wherein the *cis*-derivative of retinoic acid is selected from



the group consisting of

(MDI 301), 1-(9-cis-

retinoyloxy)-2-propanone, 1-(9-cis-retinoyloxy)-3-decanoyloxy-2-propanone, 1,3-bis-(9-cis-retinoyloxy)-2-propanone, 1-(9-cis-retinoyloxy)-2-pinacolone, 2-(9-cis-retinoyloxy)-acetophenone, 9-cis-retinoyloxy methyl 2,2-dimethyl propanoate, 2-(9-cis-retinoyloxy)-n-methyl-acetamide, 1-(9-cis-retinoyloxy)-3-hydroxy-2-propanone, 1-(9-cis-retinoyloxy)-2,3-dioleoylpropanone, and succinimidyl 9-cis-retinoate.

15

19. The method of claim 17, wherein the sample is from a human.

20

20. The method of claim 19, wherein the human is diagnosed with AML and/or PML.

21. The method of claim 17, wherein the sample is an in vivo sample.

25

22. The method of claim 17, wherein the sample is an in vitro sample.

FIG. 1A

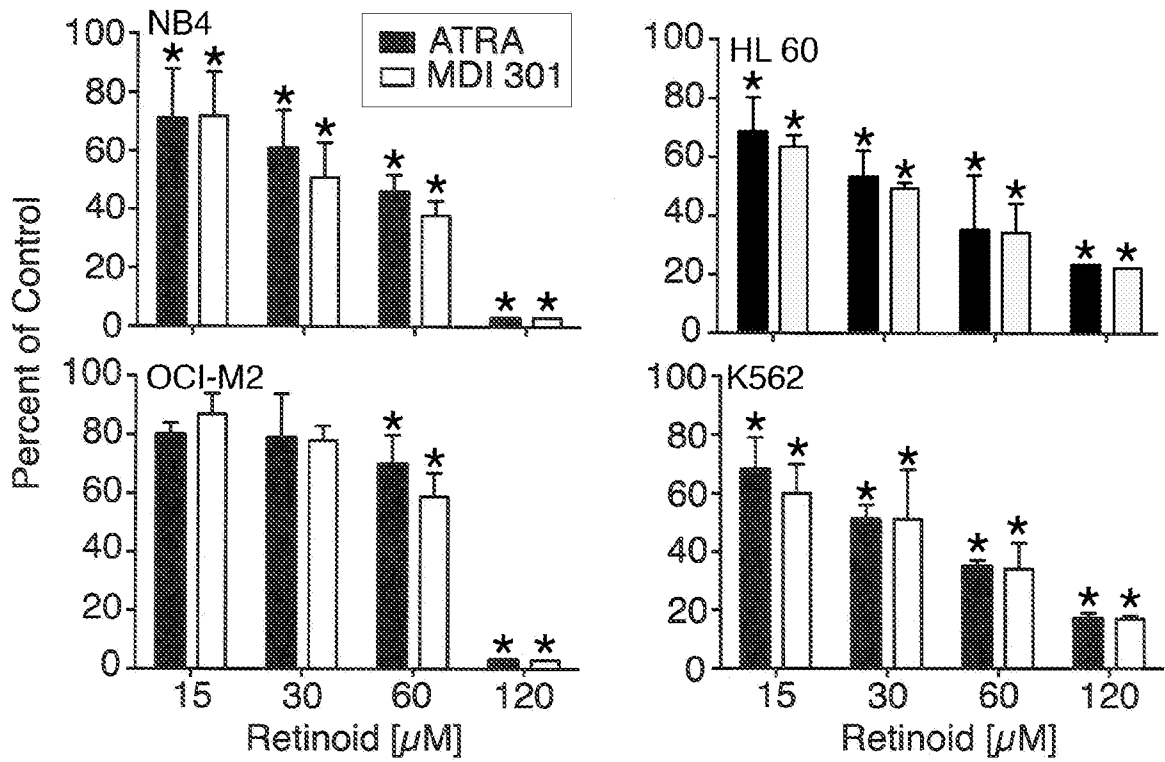


FIG. 1B

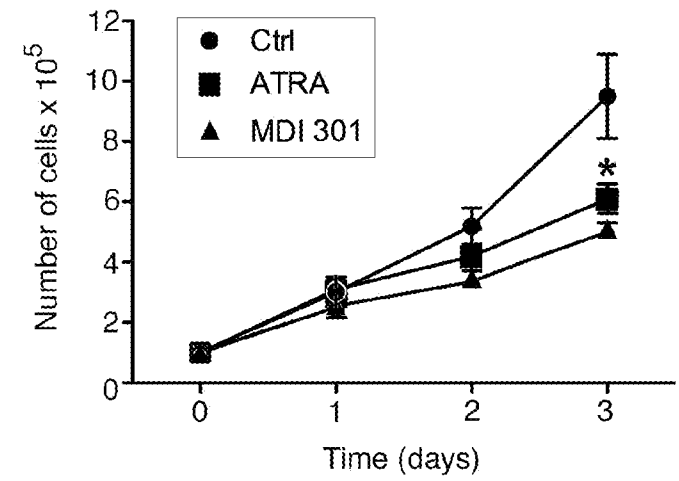


FIG. 2

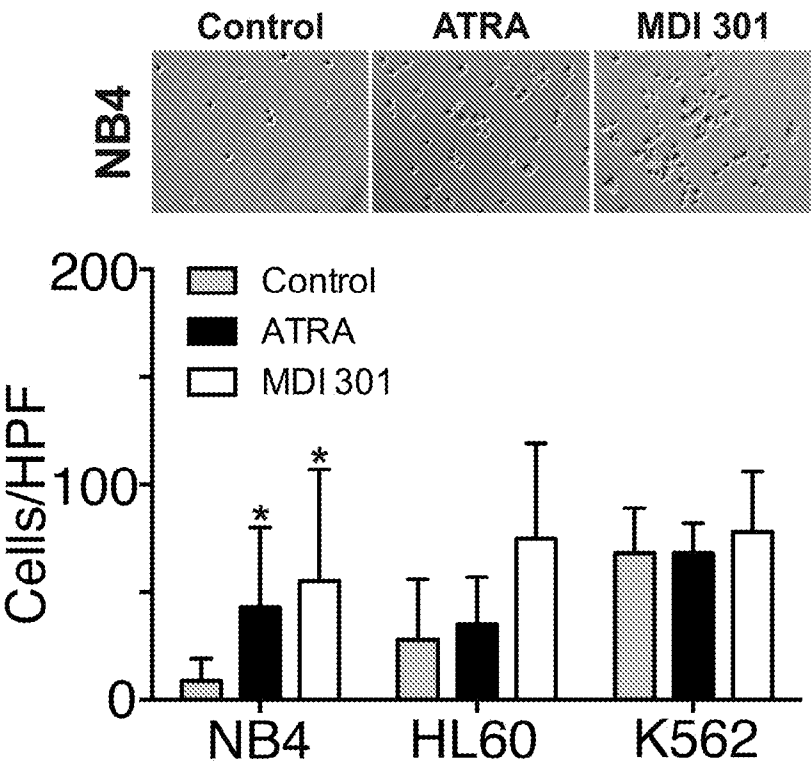


FIG. 3

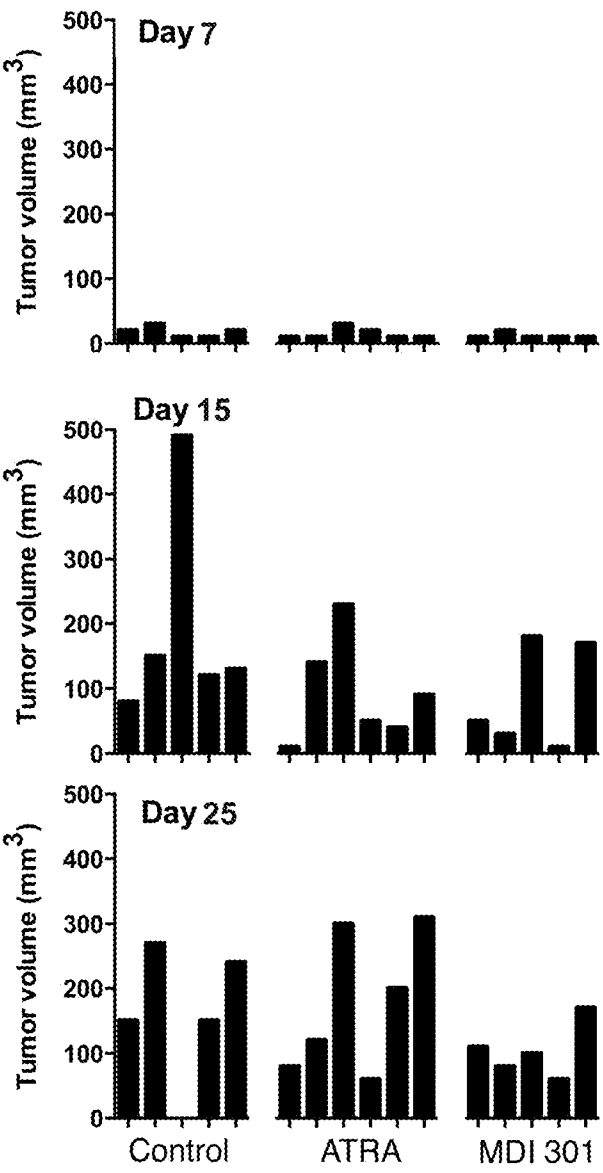
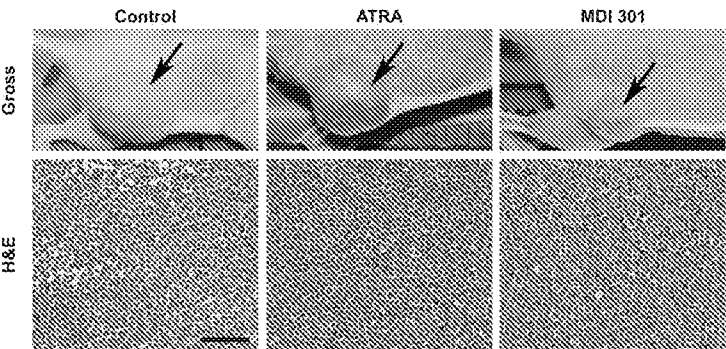


FIG. 4

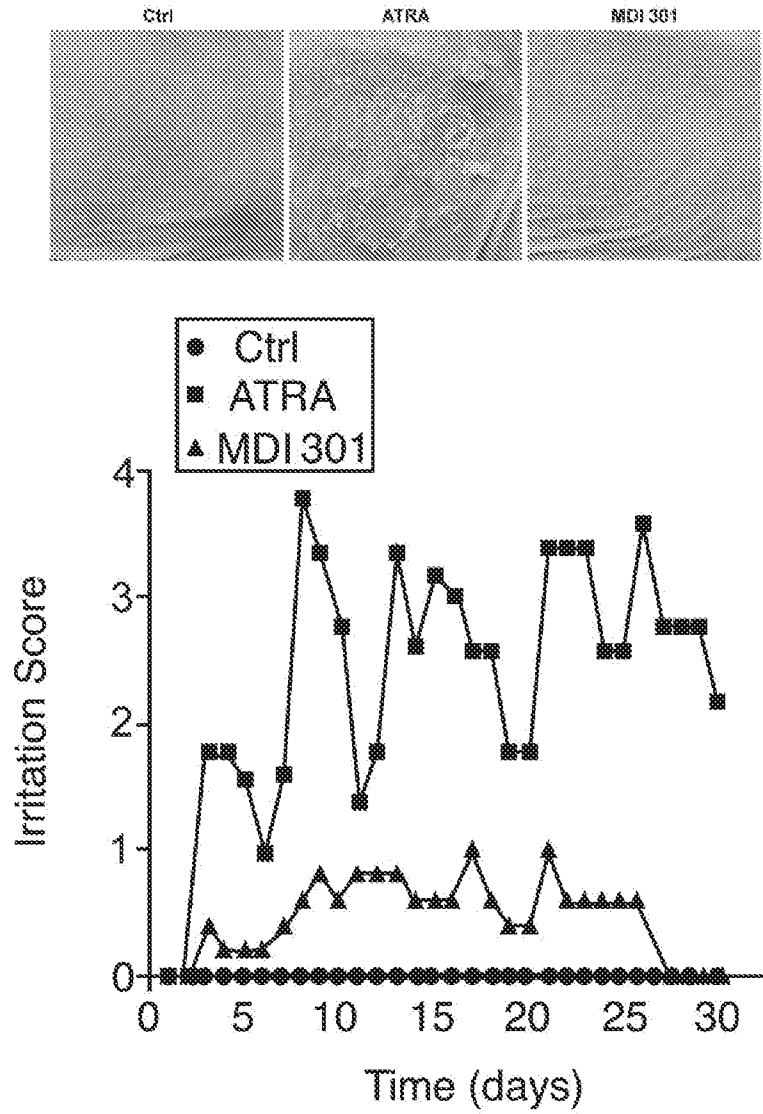
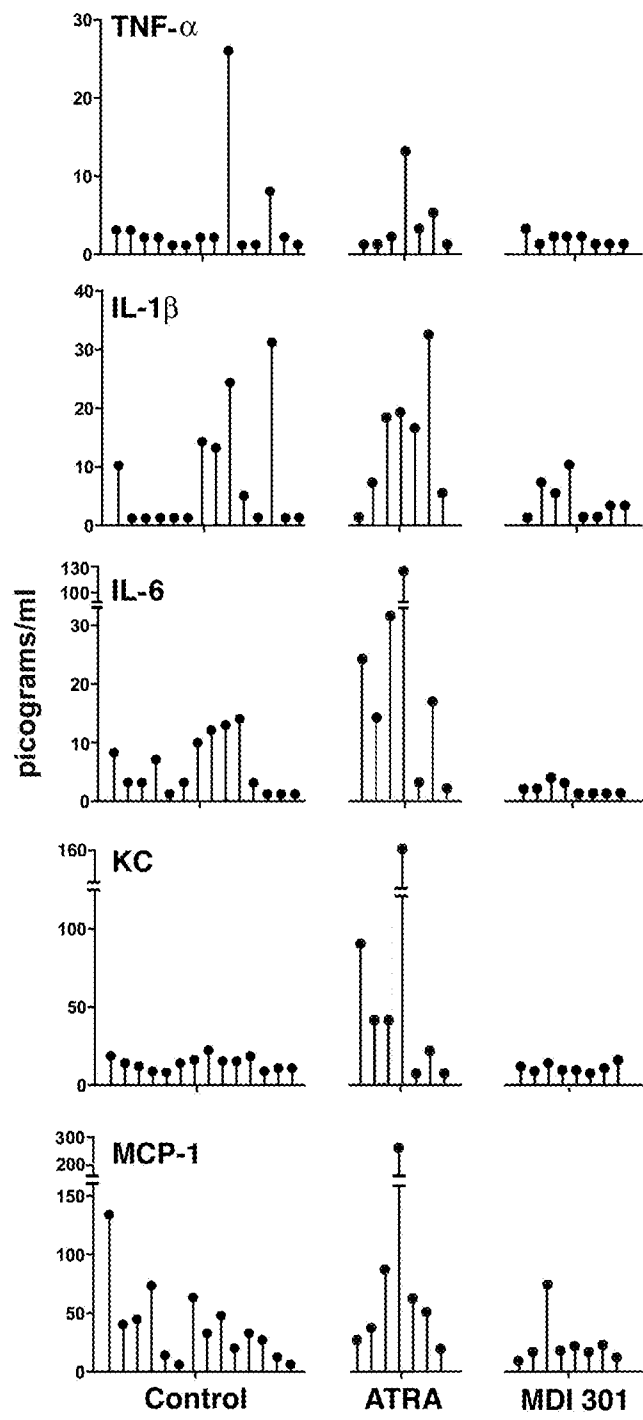


FIG. 5



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/017564

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - A61K 31/203; A61K 31/185; A61P 35/00; A61P 35/02 (2016.01) CPC - A61K 31/203; C07C 403/20; A61K 31/19; A61K 31/185; C07C 403/12; C12P 7/6418 (2016.05) According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC(8) - A61K 31/185; A61K 31/203; A61P 35/00; A61P 35/02 (2016.01) CPC - A61K 31/19; A61K 31/185; A61K 31/203; C07C 403/12; C07C 403/20; C12P 7/6418 (2016.05) Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched IPC(8) - A61K 31/185; A61K 31/203; A61P 35/00; A61P 35/02 (2016.01); CPC - A61K 31/19; A61K 31/185; A61K 31/203; C07C 403/12; C07C 403/20; C12P 7/6418 (2016.05); USPC - 514/557, 559, 725 (keyword delimited) Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PatBase, PubChem, STN, Google Patents, Google Scholar Search terms used: Retinoic acid ester picolinyl AML myeloid leukemia		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2009/0023149 A1 (KNUDSEN) 22 January 2009 (22.01.2009) entire document	1-5, 7-10, 17-22
A	PUBCHEM, Substance Record for SID 80158124, Create Date: 2009-06-12. [retrieved on 23 May 2016]. Retrieved from the Internet. < https://pubchem.ncbi.nlm.nih.gov/substance/80158124/version/1#section=Top >. entire document	1-5, 7-10, 17-22
A	US 2008/0139518 A1 (PURCELL) 12 June 2008 (12.06.2008) entire document	1-5, 7-10, 17-22
A	US 7,223,397 B1 (ROSENBLUM et al) 29 May 2007 (29.05.2007) entire document	1-5, 7-10, 17-22
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 23 May 2016		Date of mailing of the international search report 20 JUN 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300		Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/017564

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Extra Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5, 7-10, 17-22

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/017564

Continued from Box No. III Observations where unity of invention is lacking

Claims 1-5, 7-10, and 17-22 have been analyzed subject to the restriction that the claims read on the method as described in the Lack of Unity of Invention (See Box IV). The claims are restricted to a method of treating, ameliorating, or preventing recurrence of a condition involving myeloid leukemia cell growth in a patient comprising administering to said patient a therapeutically effective amount of a cis-derivative of retinoic acid, including salts, esters and prodrugs thereof, and a pharmaceutically acceptable carrier; wherein the cis-derivative of retinoic acid is the first shown structure of claim 2 (MDI 301); wherein the myeloid leukemia cell grown is AML.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-10 and 17-22 are drawn to methods of treating, ameliorating, or preventing recurrence of a condition involving myeloid leukemia cell growth.

Group II+: claims 11-16 are drawn to kits thereof.

The first invention of Group I+ is restricted to a method of treating, ameliorating, or preventing recurrence of a condition involving myeloid leukemia cell growth in a patient comprising administering to said patient a therapeutically effective amount of a cis-derivative of retinoic acid, including salts, esters and prodrugs thereof, and a pharmaceutically acceptable carrier; wherein the cis-derivative of retinoic acid is the first shown structure of claim 2 (MDI 301); wherein the myeloid leukemia cell grown is AML. It is believed that claims 1-5, 7-10, and 17-22 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

The first invention of Group II+ is restricted to a kit comprising a pharmaceutical composition comprising a cis-derivative of retinoic acid, and instructions for administering said pharmaceutical composition to a patient diagnosed with AML; wherein the cis-derivative of retinoic acid is the first shown structure of claim 2 (MDI 301).

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be a method of treating, ameliorating, or preventing recurrence of a condition involving myeloid leukemia cell growth in a patient comprising administering to said patient a therapeutically effective amount of a cis-derivative of retinoic acid, including salts, esters and prodrugs thereof, and a pharmaceutically acceptable carrier; wherein the cis-derivative of retinoic acid is succinimidyl 9-cis-retinoate; wherein the myeloid leukemia cell grown is AML. Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ and II+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I+, methods, are not present in Group II+, and the special technical features of Group II+, kits thereof, are not present in Group I+.

The Groups I+ and II+ formulae do not share a significant structural element requiring the selection of alternatives for the cis-derivative of retinoic acid and the myeloid leukemia cell growth.

The Groups I+ and II+ share the technical features of a method of treating, ameliorating, or preventing recurrence of a condition involving myeloid leukemia cell growth in a patient comprising administering to said patient a therapeutically effective amount of a cis-derivative of retinoic acid, including salts, esters and prodrugs thereof, and a pharmaceutically acceptable carrier; a kit comprising a pharmaceutical composition comprising a cis-derivative of retinoic acid, and instructions for administering said pharmaceutical composition to a patient diagnosed with AML and/or PML; and a method of inhibiting myeloid leukemia cell growth, comprising exposing to a sample comprising proliferating myeloid leukemia cells a composition comprising a cis-derivative of retinoic acid, wherein the exposing results in inhibition of myeloid leukemia cell growth. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 7,223,397 B1 to Rosenblum et al. teach a method of treating, ameliorating, or preventing recurrence of a condition involving myeloid leukemia cell growth in a patient (Abstract) comprising administering to said patient a therapeutically effective amount of a cis-derivative of retinoic acid (Col. 2, Lns. 12-32; Claims 1 and 2); including salts, esters and prodrugs thereof, and a pharmaceutically acceptable carrier (Col. 4, Ln. 60 through Col. 5, Ln. 12); and a method of inhibiting myeloid leukemia cell growth (Abstract; Col. 4, Ln. 60 through Col. 5, Ln. 12), comprising exposing to a sample comprising proliferating myeloid leukemia cells a composition (Col. 4, Ln. 60 through Col. 5, Ln. 12) comprising a cis-derivative of retinoic acid (Col. 2, Lns. 12-32; Claims 1 and 2), wherein the exposing results in inhibition of myeloid leukemia cell growth (Col. 4, Ln. 60 through Col. 5, Ln. 12).

Additionally, US 2009/0023149 A1 to Knudsen teaches a kit (Claim 203; Para. [0011]) comprising a cis-derivative of retinoic acid (Para. [0021]), in an embodiment of all aspects of the invention, the treatment is...retinoic acid, 3-Methyl TTNEB, 9-retinoic acid...), and instructions for administering said cis-derivative of retinoic acid to a patient diagnosed with AML and/or PML (Claim 203; Para. [0014], The kit further includes instructions...; Para. [0005]...acute leukemia...).

The inventions listed in Groups I+ and II+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.