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(54) Title: TREATMENT OF MULTIPLE MYELOMA WITH HETEROCYCLIC INHIBITORS OF GLUTAMINASE

(57) Abstract: The invention relates to novel heterocyclic compounds and pharmaceutical preparations thereof. The invention further relates to methods of treating or preventing multiple myeloma using the novel heterocyclic compounds of the invention.

Treatment of Multiple Myeloma with Heterocyclic Inhibitors of Glutaminase

Related Applications

This application claims the benefit of priority to U.S. Provisional Patent Application serial number 62/028,523, filed July 24, 2014, the contents of which are hereby incorporated by reference.

Background

Glutamine supports cell survival, growth and proliferation through metabolic and non-metabolic mechanisms. In actively proliferating cells, the metabolism of glutamine to lactate, also referred to as "glutaminolysis" is a major source of energy in the form of NADPH. The first step in glutaminolysis is the deamination of glutamine to form glutamate and ammonia, which is catalyzed by the glutaminase enzyme (GLS). Thus, deamination via glutaminase is a control point for glutamine metabolism.

Ever since Warburg's observation that ascites tumor cells exhibited high rates of glucose consumption and lactate secretion in the presence of oxygen (Warburg, 1956), researchers have been exploring how cancer cells utilize metabolic pathways to be able to continue actively proliferating. Several reports have demonstrated how glutamine metabolism supports macromolecular synthesis necessary for cells to replicate (Curthoys, 1995; DeBardinis, 2008).

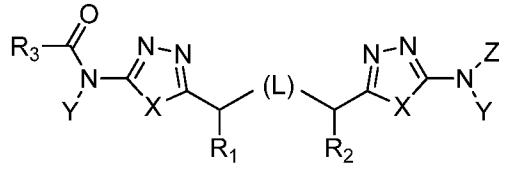
Thus, glutaminase has been theorized to be a potential therapeutic target for the treatment of diseases characterized by actively proliferating cells, such as cancer. The lack of suitable glutaminase inhibitors has made validation of this target impossible until the recent creation of compounds that are specific and capable of being formulated for in vivo use (US 8,604,016). As glutaminase inhibitors enter the clinical arena, methods are needed to identify patients that would best benefit from treatment with these compounds.

Summary of Invention

The present invention provides a method for treating multiple myeloma in a patient, comprising determining a level of pyruvate carboxylase in a patient sample comprising multiple myeloma cancer cells, comparing the level of pyruvate carboxylase in the patient sample to a reference standard, and if the level of pyruvate

carboxylase in the patient sample is lower than the reference standard, then administering to the patient an effective amount of a glutaminase inhibitor.

In some embodiments, the glutaminase inhibitor is a compound of formula I,



(I),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or  , preferably CH_2CH_2, wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X, independently for each occurrence, represents S, O or CH=CH, preferably S or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, arylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy;

R_3, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H.

In further embodiments, the invention relates to methods of identifying a multiple myeloma patient that may benefit from treatment with a glutaminase inhibitor, comprising determining the level of pyruvate carboxylase in multiple myeloma cancer cells of the patient compared to a reference standard, wherein a lower level in the cancer cells of the patient as compared to the standard indicates that the patient may benefit from treatment with a glutaminase inhibitor.

Brief Description of the Drawings

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figure 1 shows the correlation between glutamine-dependence and antiproliferative effect of compound 670 for a panel of breast tumor cell lines.

Figure 2 shows single-agent compound 402 treatment of MDA-MB-231 orthotopic xenograft model.

Figure 3 shows a combination study with compound 389 and paclitaxel in MDA-MB-231 orthotopic xenograft model.

Figure 4 shows results of the median glutaminase:glutamine synthetase expression ratio in various cancer types, including colorectal cancer, renal cancer, lymphoma, melanoma and myeloma.

Figure 5 shows that intraperitoneal administration of compound 188 to mice results in reduced tumor size in a HCT116 colon carcinoma xenograft model.

Figure 6 shows that oral administration of compound 670 to mice results in reduced tumor size in a H2122 lung adenocarcinoma xenograft model.

Figure 7 shows that oral administration of compound 670 to mice results in reduced tumor size in a RPMI-8226 multiple myeloma xenograft model.

Figure 8 shows that compound 670 synergizes with pomalidomide or dexamethasone to produce an anti-tumor effect in multiple myeloma cells.

Figure 9 shows a waterfall plot demonstrating the response variation to CB-839 treatment in a panel of multiple myeloma tumor cell lines.

Figure 10 shows a western blot for pyruvate carboxylase in a panel of multiple myeloma cell lines.

Figure 11 shows a correlation between pyruvate carboxylase protein level and cell growth in response to CB-839 treatment relative to no CB-839 treatment/DMSO control.

Figure 12 shows pyruvate carboxylase expression values generated from RNA-seq data for various multiple myeloma cell lines correlates with pyruvate carboxylase protein levels.

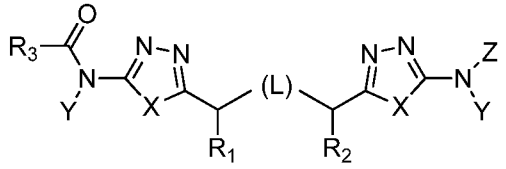
Detailed Description of the Invention

The present invention is based on the discovery of a biomarker in multiple myeloma cell lines that indicates sensitivity of the multiple myeloma cells to treatment with a glutaminase inhibitor. As shown in data presented herein, not all multiple myeloma cell lines exhibit the same sensitivity to treatment with a glutaminase inhibitor. Further, this effect is not observed in certain other types of cancer cell lines (data not shown).

The present invention provides a method for treating multiple myeloma in a patient, comprising determining a level of pyruvate carboxylase in a patient sample comprising multiple myeloma cancer cells, comparing the level of pyruvate carboxylase in the patient sample to a reference standard, and if the level of pyruvate

carboxylase in the patient sample is lower than the reference standard, then administering to the patient an effective amount of a glutaminase inhibitor.

In certain embodiments, the glutaminase inhibitor is a compound of formula I,



(I),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or  , preferably CH_2CH_2, wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X, independently for each occurrence, represents S, O or CH=CH, preferably S or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, arylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy;

R_3, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H.

In certain embodiments wherein alkyl, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl are substituted, they are substituted with one or more substituents selected from substituted or unsubstituted alkyl, such as perfluoroalkyl (e.g., trifluoromethyl), alkenyl, alkoxy, alkoxyalkyl, aryl, aralkyl, arylalkoxy, aryloxy, aryloxyalkyl, hydroxyl, halo, alkoxy, such as perfluoroalkoxy (e.g., trifluoromethoxy), alkoxyalkoxy, hydroxyalkyl, hydroxyalkylamino, hydroxyalkoxy, amino, aminoalkyl, alkylamino, aminoalkylalkoxy, aminoalkoxy, acylamino, acylaminoalkyl, such as perfluoro acylaminoalkyl (e.g., trifluoromethylacylaminoalkyl), acyloxy, cycloalkyl, cycloalkylalkyl, cycloalkylalkoxy, heterocyclyl, heterocyclylalkyl, heterocyclyloxy, heterocyclylalkoxy, heteroaryl, heteroarylalkyl, heteroarylalkoxy, heteroaryloxy, heteroaryloxyalkyl, heterocyclylaminoalkyl, heterocyclylaminoalkoxy, amido, amidoalkyl, amidine, imine, oxo, carbonyl (such as carboxyl, alkoxycarbonyl, formyl, or acyl, including perfluoroacyl (e.g., $C(O)CF_3$)), carbonylalkyl (such as carboxyalkyl, alkoxycarbonylalkyl, formylalkyl, or acylalkyl, including perfluoroacylalkyl (e.g., -alkyl$C(O)CF_3$)), carbamate, carbamatealkyl, urea, ureaalkyl, sulfate, sulfonate, sulfamoyl, sulfone, sulfonamide, sulfonamidealkyl, cyano, nitro,

azido, sulfhydryl, alkylthio, thiocarbonyl (such as thioester, thioacetate, or thioformate), phosphoryl, phosphate, phosphonate or phosphinate.

In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, or CH_2NHCH_2, wherein any hydrogen atom of a CH_2 unit may be replaced by alkyl or alkoxy, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxyl. In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2. In certain embodiments, L represents CH_2CH_2. In certain embodiments, L is not CH_2SCH_2.

In certain embodiments, Y represents H.

In certain embodiments, X represents S or CH=CH. In certain embodiments, one or both X represents CH=CH. In certain embodiments, each X represents S. In certain embodiments, one X represents S and the other X represents CH=CH.

In certain embodiments, Z represents $R_3(CO)$. In certain embodiments wherein Z is $R_3(CO)$, each occurrence of R_3 is not identical (e.g., the compound of formula I is not symmetrical).

In certain embodiments, R_1 and R_2 each represent H.

In certain embodiments, R_3 represents arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl. In certain embodiments, R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents aryl, arylalkyl, heteroaryl or heteroaralkyl, such as aryl, arylalkyl or heteroaryl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl, such as hydroxy, hydroxyalkyl or alkoxy.

In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, such as CH_2CH_2, CH_2S or SCH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and each R_3 represents arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl. In certain such embodiments, each occurrence of R_3 is identical.

In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and each R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents aryl, arylalkyl, heteroaryl or heteroaralkyl, such as aryl, arylalkyl or heteroaryl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl, such as hydroxy, hydroxyalkyl or alkoxy. In certain such embodiments, each occurrence of R_3 is identical.

In certain embodiments, L represents CH_2CH_2, Y represents H, X represents S or CH=CH, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and each R_3 represents

substituted or unsubstituted arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl. In certain such embodiments, each X represents S. In other embodiments, one or both occurrences of X represents CH=CH, such as one occurrence of X represents S and the other occurrence of X represents CH=CH. In certain embodiments of the foregoing, each occurrence of R_3 is identical. In other embodiments of the foregoing wherein one occurrence of X represents S and the other occurrence of X represents CH=CH, the two occurrences of R_3 are not identical.

In certain embodiments, L represents CH_2CH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and each R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents aryl, arylalkyl or heteroaryl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl or alkoxy. In certain such embodiments, R_8 represents aryl and R_{10} represents hydroxyalkyl. In certain such embodiments, each occurrence of R_3 is identical.

In certain embodiments wherein L represents CH_2, $CH_2CH_2CH_2$ or CH_2CH_2, X represents O, and Z represents $R_3(CO)$, both R_3 groups are not alkyl, such as methyl, or $C(R_8)(R_9)(R_{10})$, wherein R_8, R_9 and R_{10} are each independently hydrogen or alkyl.

In certain embodiments wherein L represents CH_2CH_2, X represents S, and Z represents $R_3(CO)$, both R_3 groups are not phenyl or heteroaryl, such as 2-furyl.

In certain embodiments wherein L represents CH_2CH_2, X represents O, and Z represents $R_3(CO)$, both R_3 groups are not $N(R_4)(R_5)$ wherein R_4 is aryl, such as phenyl, and R_5 is H.

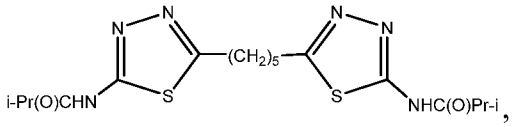
In certain embodiments wherein L represents CH_2SCH_2, X represents S, and Z represents $R_3(CO)$, both R_3 groups are not aryl, such as optionally substituted phenyl, aralkyl, such as benzyl, heteroaryl, such as 2-furyl, 2-thienyl or 1,2,4-trizole, substituted or unsubstituted alkyl, such as methyl, chloromethyl, dichloromethyl, n-propyl, n-butyl, t-butyl or hexyl, heterocyclyl, such as pyrimidine-2,4(1H,3H)-dione, or alkoxy, such as methoxy, pentyloxy or ethoxy.

In certain embodiments wherein L represents CH_2SCH_2, X represents S, and Z represents $R_3(CO)$, both R_3 groups are not $N(R_4)(R_5)$ wherein R_4 is aryl, such as substituted or unsubstituted phenyl (e.g., phenyl, 3-tolyl, 4-tolyl, 4-bromophenyl or 4-nitrophenyl), and R_5 is H.

In certain embodiments wherein L represents $CH_2CH_2CH_2$, X represents S, and Z represents $R_3(CO)$, both R_3 groups are not alkyl, such as methyl, ethyl, or

propyl, cycloalkyl, such as cyclohexyl, or $C(R_8)(R_9)(R_{10})$, wherein any of R_8, R_9 and R_{10} together with the C to which they are attached, form any of the foregoing.

In certain embodiments, the compound is not one of the following:



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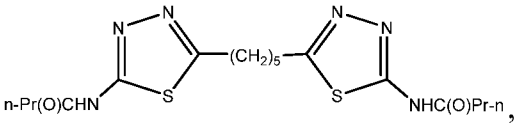

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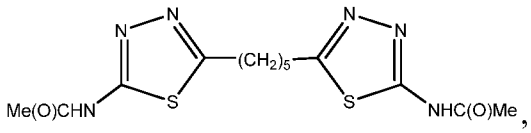

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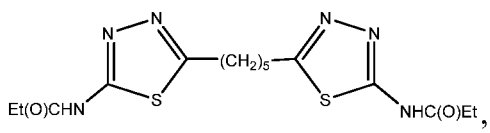

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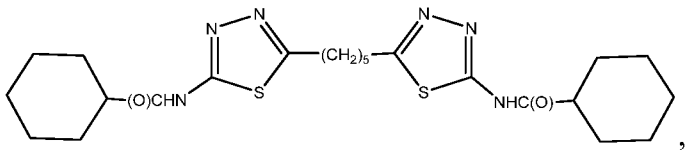

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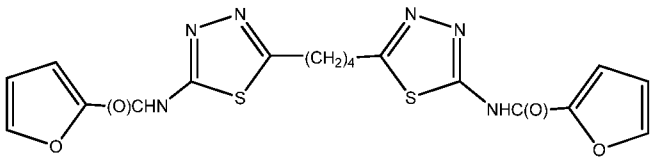

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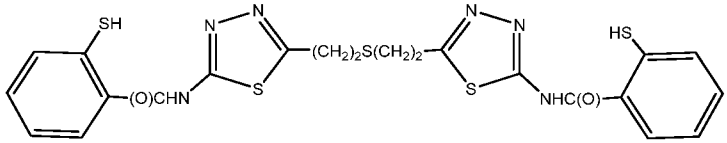

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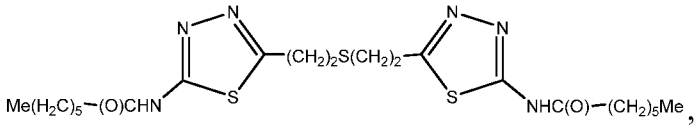

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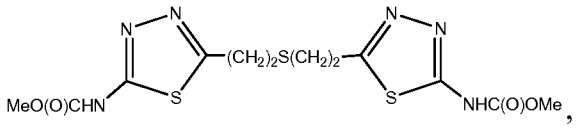

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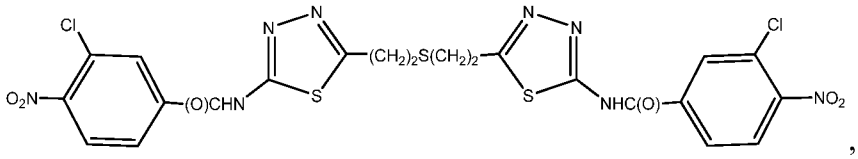

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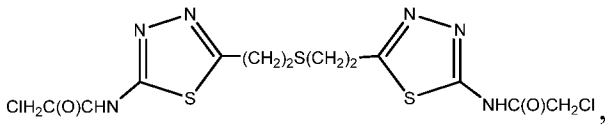

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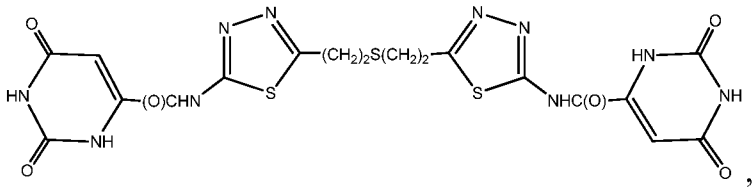

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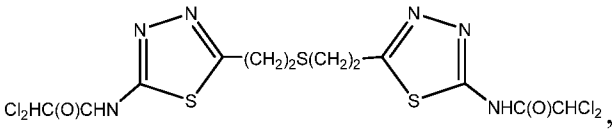

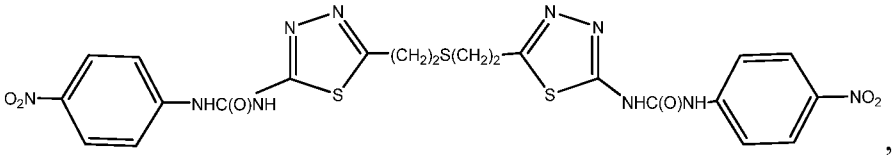

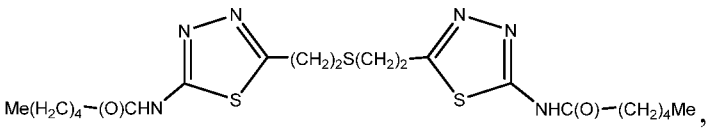

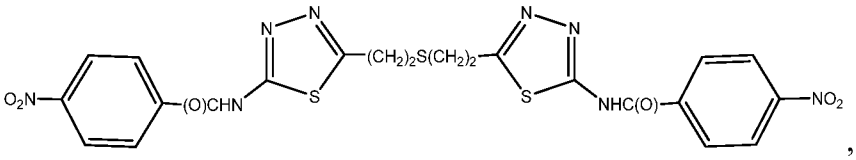

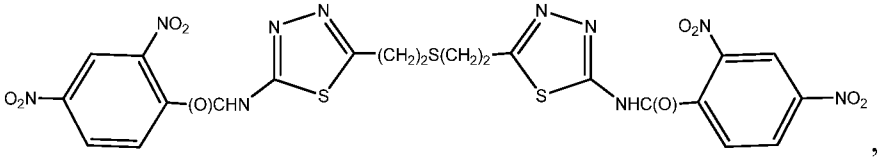

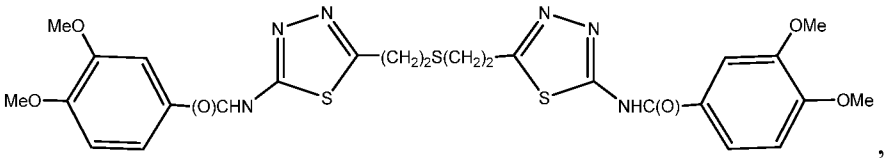

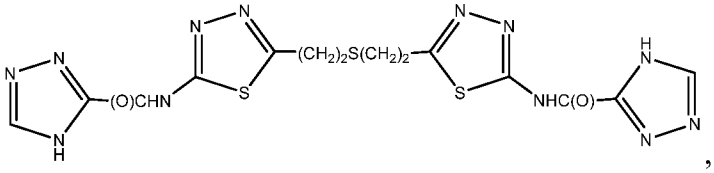

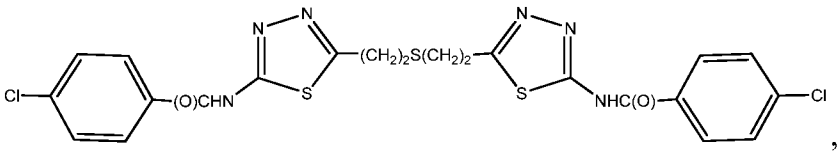

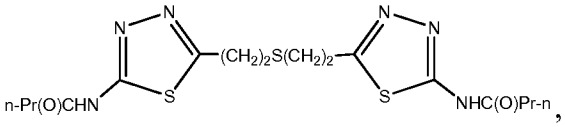

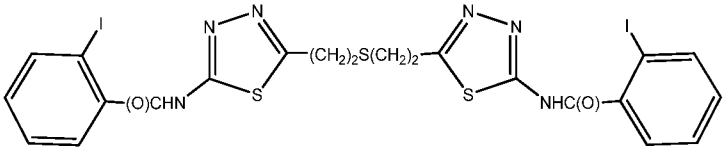

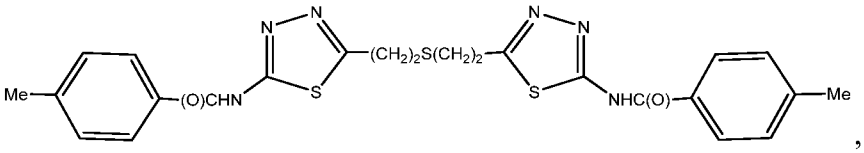

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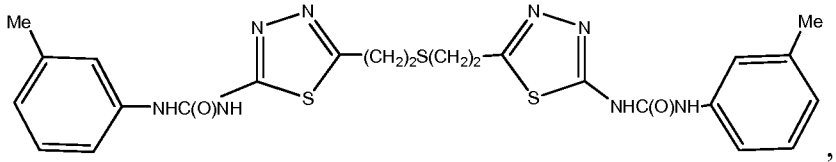
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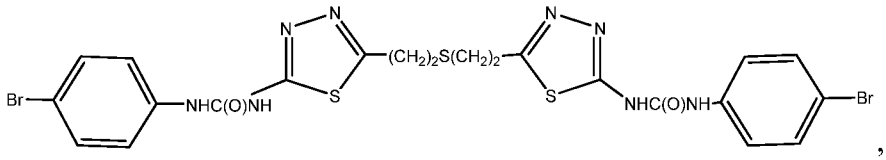
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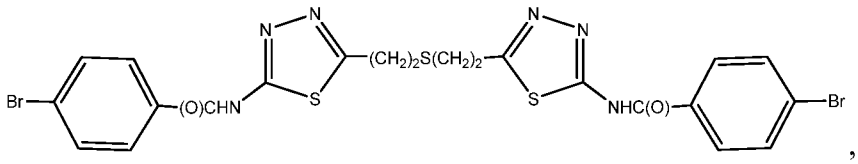
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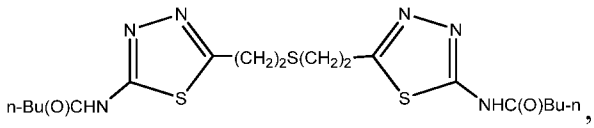
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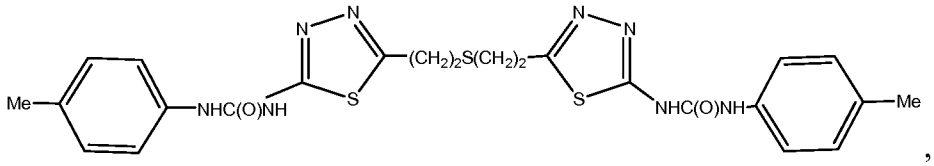
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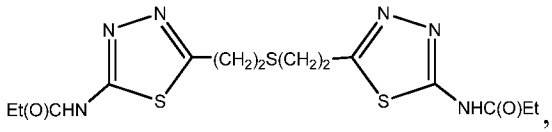
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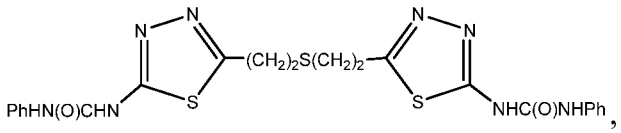
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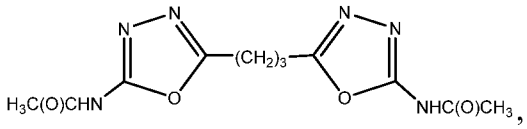
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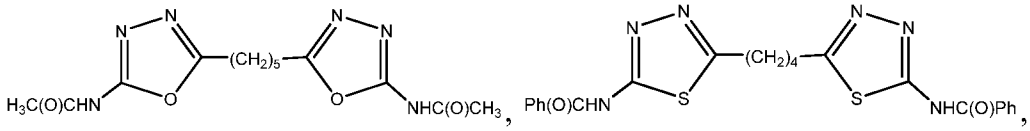
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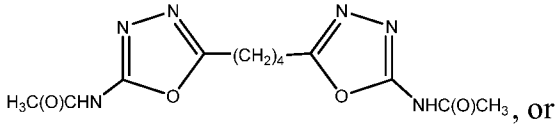
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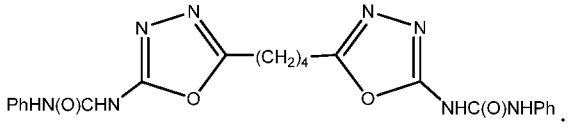
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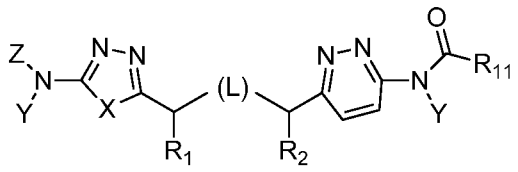
In further embodiments of the methods of the invention, the glutaminase inhibitor is a compound of formula Ia,



(Ia),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or  , preferably CH_2CH_2, wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X represents S, O or CH=CH, preferably S or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, arylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy, preferably H;

R_3 represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl,

heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H;

R_{11} represents substituted or unsubstituted aryl, arylalkyl, aryloxy, aryloxyalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or $C(R_{12})(R_{13})(R_{14})$, $N(R_4)(R_{14})$ or OR_{14}, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_{12} and R_{13} each independently respresent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein both of R_{12} and R_{13} are not H; and

R_{14} represents substituted or unsubstituted aryl, arylalkyl, aryloxy, aryloxyalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl.

In certain embodiments wherein alkyl, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl are substituted, they are substituted with one or more substituents selected from substituted or unsubstituted alkyl, such as perfluoroalkyl (e.g., trifluoromethyl), alkenyl, alkoxy, alkoxyalkyl, aryl, aralkyl, arylalkoxy, aryloxy, aryloxyalkyl, hydroxyl, halo, alkoxy, such as perfluoroalkoxy (e.g., trifluoromethylalkoxy), alkoxyalkoxy, hydroxyalkyl, hydroxyalkylamino, hydroxyalkoxy, amino, aminoalkyl, alkylamino,

aminoalkylalkoxy, aminoalkoxy, acylamino, acylaminoalkyl, such as perfluoro acylaminoalkyl (e.g., trifluoromethylacylaminoalkyl), acyloxy, cycloalkyl, cycloalkylalkyl, cycloalkylalkoxy, heterocyclyl, heterocyclylalkyl, heterocyclyloxy, heterocyclylalkoxy, heteroaryl, heteroarylalkyl, heteroarylalkoxy, heteroaryloxy, heteroaryloxyalkyl, heterocyclylaminoalkyl, heterocyclylaminoalkoxy, amido, amidoalkyl, amidine, imine, oxo, carbonyl (such as carboxyl, alkoxycarbonyl, formyl, or acyl, including perfluoroacyl (e.g., $C(O)CF_3$)), carbonylalkyl (such as carboxyalkyl, alkoxycarbonylalkyl, formylalkyl, or acylalkyl, including perfluoroacylalkyl (e.g., -alkyl$C(O)CF_3$)), carbamate, carbamatealkyl, urea, ureaalkyl, sulfate, sulfonate, sulfamoyl, sulfone, sulfonamide, sulfonamidealkyl, cyano, nitro, azido, sulfhydryl, alkylthio, thiocarbonyl (such as thioester, thioacetate, or thioformate), phosphoryl, phosphate, phosphonate or phosphinate.

In certain embodiments, R_{11} represents substituted or unsubstituted arylalkyl, such as substituted or unsubstituted benzyl.

In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, or CH_2NHCH_2, wherein any hydrogen atom of a CH_2 unit may be replaced by alkyl or alkoxy, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxyl. In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, preferably CH_2CH_2. In certain embodiments, L is not CH_2SCH_2.

In certain embodiments, each Y represents H. In other embodiments, at least one Y is $CH_2O(CO)R_7$.

In certain embodiments, X represents S or CH=CH. In certain embodiments, X represents S.

In certain embodiments, R_1 and R_2 each represent H.

In certain embodiments, Z represents $R_3(CO)$. In certain embodiments wherein Z is $R_3(CO)$, R_3 and R_{11} are not identical (e.g., the compound of formula I is not symmetrical).

In certain embodiments, Z represents $R_3(CO)$ and R_3 represents arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl. In certain embodiments, Z represents $R_3(CO)$ and R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents aryl, arylalkyl, heteroaryl or heteroaralkyl, such as aryl, arylalkyl or heteroaryl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl, such as hydroxy,

hydroxyalkyl or alkoxy. In certain embodiments, Z represents $R_3(CO)$ and R_3 represents heteroarylalkyl.

In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, such as CH_2CH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, R_3 represents arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl, and R_{11} represents arylalkyl. In certain such embodiments, R_3 represents heteroarylalkyl.

In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, such as CH_2CH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents aryl, arylalkyl, heteroaryl or heteroaralkyl, such as aryl, arylalkyl or heteroaryl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl, such as hydroxy, hydroxyalkyl or alkoxy, and R_{11} represents arylalkyl. In certain such embodiments, R_8 represents heteroaryl.

In certain embodiments, L represents CH_2CH_2, Y represents H, X represents S or CH=CH, such as S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, R_3 represents substituted or unsubstituted arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl, and R_{11} represents arylalkyl. In certain such embodiments, R_3 represents heteroarylalkyl.

In certain embodiments, L represents CH_2CH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents aryl, arylalkyl or heteroaryl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl or alkoxy, and R_{11} represents arylalkyl. In certain such embodiments, R_8 represents aryl and R_{10} represents hydroxyalkyl. In certain other embodiments, R_8 represents heteroaryl.

In certain embodiments, compounds of the invention may be prodrugs of the compounds of formula I or Ia, e.g., wherein a hydroxyl in the parent compound is presented as an ester or a carbonate, or carboxylic acid present in the parent compound is presented as an ester. In certain such embodiments, the prodrug is metabolized to the active parent compound in vivo (e.g., the ester is hydrolyzed to the corresponding hydroxyl, or carboxylic acid).

In certain embodiments, compounds of the invention may be racemic. In certain embodiments, compounds of the invention may be enriched in one enantiomer. For example, a compound of the invention may have greater than 30%

ee, 40% ee, 50% ee, 60% ee, 70% ee, 80% ee, 90% ee, or even 95% or greater ee. In certain embodiments, compounds of the invention may have more than one stereocenter. In certain such embodiments, compounds of the invention may be enriched in one or more diastereomer. For example, a compound of the invention may have greater than 30% de, 40% de, 50% de, 60% de, 70% de, 80% de, 90% de, or even 95% or greater de.

In certain embodiments, the present invention relates to methods of treating or preventing multiple myeloma with a compound of formula I or Ia, or a pharmaceutically acceptable salt thereof. In certain embodiments, the therapeutic preparation may be enriched to provide predominantly one enantiomer of a compound (e.g., of formula I or Ia). An enantiomerically enriched mixture may comprise, for example, at least 60 mol percent of one enantiomer, or more preferably at least 75, 90, 95, or even 99 mol percent. In certain embodiments, the compound enriched in one enantiomer is substantially free of the other enantiomer, wherein substantially free means that the substance in question makes up less than 10%, or less than 5%, or less than 4%, or less than 3%, or less than 2%, or less than 1% as compared to the amount of the other enantiomer, *e.g.*, in the composition or compound mixture. For example, if a composition or compound mixture contains 98 grams of a first enantiomer and 2 grams of a second enantiomer, it would be said to contain 98 mol percent of the first enantiomer and only 2% of the second enantiomer.

In certain embodiments, the therapeutic preparation may be enriched to provide predominantly one diastereomer of a compound (e.g., of formula I or Ia). A diastereomerically enriched mixture may comprise, for example, at least 60 mol percent of one diastereomer, or more preferably at least 75, 90, 95, or even 99 mol percent.

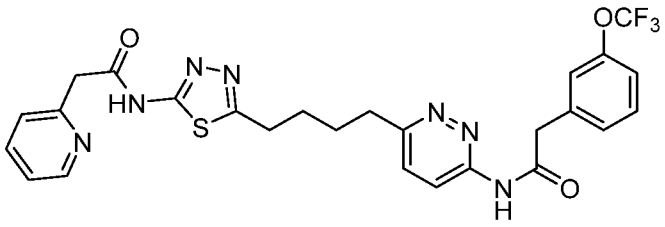
In certain embodiments, the present invention provides a pharmaceutical preparation suitable for use in a human patient in the treatment of multiple myeloma, comprising an effective amount of any of the compounds shown above (e.g., a compound of the invention, such as a compound of formula I or Ia), and one or more pharmaceutically acceptable excipients. In certain embodiments, the pharmaceutical preparations may be for use in treating or preventing a condition or disease as described herein. In certain embodiments, the pharmaceutical preparations have a low enough pyrogen activity to be suitable for use in a human patient.

Compounds of any of the above structures may be used in the manufacture of medicaments for the treatment of any diseases or conditions disclosed herein.

In further embodiments, the invention relates to methods of identifying a multiple myeloma patient that may benefit from treatment with a glutaminase inhibitor, comprising determining the level of pyruvate carboxylase in multiple myeloma cancer cells of the patient compared to a reference standard, wherein a lower level in the cancer cells of the patient as compared to the standard indicates that the patient may benefit from treatment with a glutaminase inhibitor.

In certain embodiments, the glutaminase inhibitor is a compound of formula I or formula Ia.

Methods of the invention include a step of comparing the level of pyruvate carboxylase in a multiple myeloma patient sample to a reference standard. In certain embodiments, the reference standard is a level of pyruvate carboxylase in a multiple myeloma cell line that is resistant to a compound of formula (II), also referred to herein as CB-839:



(II).

In certain embodiments, the multiple myeloma cell line of the reference standard is KMS-28PE or KMS-11 cells.

Uses of enzyme inhibitors

Glutamine plays an important role as a carrier of nitrogen, carbon, and energy. It is used for hepatic urea synthesis, for renal ammoniagenesis, for gluconeogenesis, and as respiratory fuel for many cells. Cells get their glutamine by either synthesizing it internally via an enzyme called glutamine synthetase (GS) or exogenously from the environment.

The conversion of glutamine into glutamate is initiated by the mitochondrial enzyme, glutaminase. There are two major forms of the enzyme, K-type and L-type, which are distinguished by their Km values for glutamine and response to glutamate, wherein the Km value, or Michaelis constant, is the concentration of substrate required to reach half the maximal velocity. The L-type, also known as "liver-type"

or GLS2, has a high Km for glutamine and is glutamate resistant. The K-type, also known as "kidney-type" or GLS1 or "KGA", has a low Km for glutamine and is inhibited by glutamate. An alternative splice form of GLS1, referred to as glutaminase C or "GAC", has recently been identified.

In addition to serving as the basic building blocks of protein synthesis, amino acids have been shown to contribute to many processes critical for growing and dividing cells, and this is particularly true for cancer cells. Nearly all definitions of cancer include reference to dysregulated proliferation. Numerous studies on glutamine metabolism in cancer indicate that many tumors are avid glutamine consumers (Souba, Ann. Surg., 1993; Collins et al., J. Cell. Physiol., 1998; Medina, J. Nutr., 2001; Shanware et al., J. Mol. Med., 2011), and this includes, but not limited to breast cancer, renal cell carcinoma, glioma, pancreatic ductal adenocarcinoma and non-small cell lung cancer. Certain embodiments of the invention relate to the use of the compounds described herein for the treatment of these cancers.

While many cancer cells depend on exogenous glutamine for survival, the degree of glutamine dependence among tumor cell subtypes may make a population of cells more susceptible to the reduction of glutamine. As an example, gene expression analysis of breast cancers has identified five intrinsic subtypes (luminal A, luminal B, basal, HER2+, and normal-like) (Sorlie et al., Proc Natl Acad Sci USA, 2001). Although glutamine deprivation has an impact on cell growth and viability, basal-like cells appear to be more sensitive to the reduction of exogenous glutamine (Kung et al., PLoS Genetics, 2011). This supports the concept that glutamine is a very important energy source in basal-like breast cancer cell lines, and suggests that inhibition of the glutaminase enzyme would be beneficial in the treatment of breast cancers comprised of basal-like cells. Figure 1 further supports the correlation that cells dependent on exogenous glutamine are susceptible to the presence of a glutaminase inhibitor in a series of breast cancer cell lines. A xenograft of glutamine dependent cells, MD-MB-231, demonstrated a sensitivity to glutaminase inhibition (Figure 2). Interestingly, the xenograft model showed some variablility in the sensitivity to glutaminase inhibiton; however, the sensitivity could be enhanced further when combined with another agent (Figure 3).

Enzyme expression levels can be determined in multiple manners, and quantitation is relative, based on a specific standard for each assay. The results can be used to provide a genetic profile, where the levels of certain genes, mRNAs or

resulting expression products form a signature pattern that can used to characterize cell types. Kung et al, demonstrated that the basal-like breast cancer cells that showed glutamine dependency exhibited a genetic profile in which GLS expression was relatively high and GS expression was relatively low. Furthermore, the expression level of GLS2 was relatively low. Analysis of primary breast tumors mRNA expression dataset (The Cancer Genome Atlas; N=756) support that basal-type cells generally have high GLS expression relative to GS expression.

This led to the hypothesis that the high GLS expression and low GS expression profile may serve as a genetic signature to identify other cancers that may be particularly dependent on exogenous glutamine, and therefore susceptible to glutaminase inhibition. Upon analysis of a vast number of primary human cancers from a commercial database, several cancers exhibited high GLS to low GS expression patterns. In addition to the breast cancers previously noted, colorectal cancer, lung cancer, melanoma, mesothelioma, renal cancer and B cell malignancies had notably high GLS/GS ratios (Figure 4). Certain embodiments of the invention relate to the use of the compounds described herein for the treatment of cancers selected from colorectal cancer, endocrine cancer, lung cancer, melanoma, mesothelioma, renal cancer and B cell malignancies. Certain embodiments of the invention relate to the use of the compounds described herein for the treatment of multiple myeloma, leukemia (including acute lymphoblastic leukemia (ALL) and chronic lymphoblastic leukemia (CLL)), or lymphoma (including Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma and Hodgkin's lymphoma).

Xenograft models of colon cancer, lung cancer and multiple myeloma showed sensitivity to treatment with a glutaminase inhibitor (Figures 5, 6, and 7), further supporting the concept that certain cancers may prove to be more responsive to treatment with glutaminase inhibitors. It was decided to further examine the dependence of multiple myeloma cells on exogenous glutamine and the sensitivity to glutaminase inhibition.

Similar to observations in breast cancer cell lines, multiple myeloma cell lines demonstrated varied sensitivity to glutaminase inhibition (Figure 9).

Given this variability in a multiple myeloma's sensitivity to glutaminase inhibition, identifying the multiple myeloma patients that would most likely benefit from treatment with a glutaminase inhibitor would be advantageous.

Several groups have suggested that glucose can also serve as a source of carbon via the conversion of pyruvate into the TCA cycle intermediate oxaloacetate by the enzyme pyruvate carboxylase (PC). The expression levels of PC across a panel of multiple myeloma cell lines with varying sensitivity to glutaminase inhibiton was examined (Figure 10). Strikingly, PC expression was higher in multiple myeloma cell lines resistant to glutaminase inhibition, while PC expression was low across most sensitive cell lines. Quantification of PC protein levels further supported this correlation to sensitivity to glutaminase inhibition (Figure 11). The correlation is maintained using alternative means of measuring PC expression levels (Figure 12).

In certain embodiments, the invention provides a method of identifying a multiple myeloma patient that may benefit from treatment with a glutaminase inhibitor, comprising determining the level of pyruvate carboxylase in multiple myeloma cancer cells of the patient compared to a reference standard, wherein a lower level in the cancer cells of the patient indicates that the patient may benefit from treatment with a glutaminase inhibitor. In certain embodiments, the invention provides a method of identifying a multiple myeloma patient that may benefit from treatment with a glutaminase inhibitor, comprising determining the level of pyruvate carboxylase in multiple myeloma cancer cells of the patient compared to a reference standard, wherein a lower level in the cancer cells of the patient as compared to the standard indicates that the patient may benefit from treatment with a glutaminase inhibitor. In some embodiments, the multiple myeloma reference standard is normal tissue from the same patient. In certain embodiments, the multiple myeloma cell line of the reference standard is KMS-28PE or KMS-11 cells. In certain embodiments the glutaminase inhibitor is a compound of formula I. In other embodiments, the glutaminase inhibitor is a compound of formula Ia.

In certain embodiments, the invention provides a method of treating a multiple myeloma patient, the method comprising determining a level of pyruvate carboxylase in a patient sample comprising multiple myeloma cancer cells, comparing the level of pyruvate carboxylase in the patient sample to a reference standard, and if the the level of pyruvate carboxylase in the patient sample is lower than the reference standard, then administering to the patient an effective amount of glutaminase inhibitor. In certain embodiments, the multiple myeloma reference standard is normal tissue from the same patient. In certain embodiments, the multiple myeloma cell line of the reference standard is KMS-28PE or KMS-11 cells. In certain embodiments the

glutaminase inhibitor is a compound of formula I. In other embodiments, the glutaminase inhibitor is a compound of formula Ia.

In some embodiments, the method of treating or preventing cancer, such as multiple myeloma, may comprise administering a compound of the invention conjointly with one or more other chemotherapeutic agent(s). Chemotherapeutic agents that may be conjointly administered with compounds of the invention include: ABT-263, aminoglutethimide, amsacrine, anastrozole, asparaginase, azacitidine, AZD5363, Bacillus Calmette–Guérin vaccine (bcg), bicalutamide, bleomycin, bortezomib, buserelin, busulfan, campothecin, capecitabine, carboplatin, carfilzomib, carmustine, chlorambucil, chloroquine, cisplatin, cladribine, clodronate, cobimetinib, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, demethoxyviridin, dexamethasone, dichloroacetate, dienestrol, diethylstilbestrol, docetaxel, doxorubicin, epirubicin, eribulin, erlotinib, estradiol, estramustine, etoposide, everolimus, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil and 5-fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, ixabepilone, lenalidomide, letrozole, leucovorin, leuprolide, levamisole, lomustine, lonidamine, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, metformin, methotrexate, mitomycin, mitotane, mitoxantrone, mutamycin, MK-2206, nilutamide, nocodazole, octreotide, oxaliplatin, olaparib, paclitaxel, pamidronate, pentostatin, perifosine, PF-04691502, plicamycin, pomalidomide, porfimer, procarbazine, raltitrexed, rituximab, romidepsin, rucaparib, selumetinib, sorafenib, streptozocin, sunitinib, suramin, talazoparib, tamoxifen, temozolomide, temsirolimus, teniposide, testosterone, thalidomide, thioguanine, thiotepa, titanocene dichloride, topotecan, trametinib, trastuzumab, tretinoin, veliparib, vinblastine, vincristine, vindesine, vinorelbine, and vorinostat (SAHA). For example, chemotherapeutic agents that may be conjointly administered with compounds of the invention include: aminoglutethimide, amsacrine, anastrozole, asparaginase, bcg, bicalutamide, bleomycin, bortezomib, buserelin, busulfan, campothecin, capecitabine, carboplatin, carfilzomib, carmustine, chlorambucil, chloroquine, cisplatin, cladribine, clodronate, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, demethoxyviridin, dichloroacetate, dienestrol, diethylstilbestrol, docetaxel, doxorubicin, epirubicin, estradiol, estramustine, etoposide, everolimus,

exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil, fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, lenalidomide, letrozole, leucovorin, leuprolide, levamisole, lomustine, lonidamine, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, metformin, methotrexate, mitomycin, mitotane, mitoxantrone, nilutamide, nocodazole, octreotide, oxaliplatin, paclitaxel, pamidronate, pentostatin, perifosine, plicamycin, pomalidomide, porfimer, procarbazine, raltitrexed, rituximab, sorafenib, streptozocin, sunitinib, suramin, tamoxifen, temozolomide, temsirolimus, teniposide, testosterone, thalidomide, thioguanine, thiotepa, titanocene dichloride, topotecan, trastuzumab, tretinoin, vinblastine, vincristine, vindesine, and vinorelbine. In other embodiments, chemotherapeutic agents that may be conjointly administered with compounds of the invention include: ABT-263, dexamethasone, 5-fluorouracil, PF-04691502, romidepsin, and vorinostat (SAHA). In certain embodiments of the methods of the invention described herein, the chemotherapeutic agent conjointly administered with compounds of the invention is a taxane chemotherapeutic agent, such as paclitaxel or docetaxel. In certain embodiments of the methods of the invention described herein, the chemotherapeutic agent conjointly administered with compounds of the invention is doxorubicin. In certain embodiments of the methods of the invention described herein, a compound of the invention is administered conjointly with a taxane chemotherapeutic agent (e.g., paclitaxel) and doxorubicin.

Many combination therapies have been developed for the treatment of cancer. In certain embodiments, compounds of the invention may be conjointly administered with a combination therapy. Examples of combination therapies with which compounds of the invention may be conjointly administered are included in Table 1.

Table 1: Exemplary combinatorial therapies for the treatment of cancer.

Name	**Therapeutic agents**
ABV	Doxorubicin, Bleomycin, Vinblastine
ABVD	Doxorubicin, Bleomycin, Vinblastine, Dacarbazine
AC (Breast)	Doxorubicin, Cyclophosphamide
AC (Sarcoma)	Doxorubicin, Cisplatin
AC (Neuroblastoma)	Cyclophosphamide, Doxorubicin
ACE	Cyclophosphamide, Doxorubicin, Etoposide

Name	Therapeutic agents
ACe	Cyclophosphamide, Doxorubicin
AD	Doxorubicin, Dacarbazine
AP	Doxorubicin, Cisplatin
ARAC-DNR	Cytarabine, Daunorubicin
B-CAVe	Bleomycin, Lomustine, Doxorubicin, Vinblastine
BCVPP	Carmustine, Cyclophosphamide, Vinblastine, Procarbazine, Prednisone
BEACOPP	Bleomycin, Etoposide, Doxorubicin, Cyclophosphamide, Vincristine, Procarbazine, Prednisone, Filgrastim
BEP	Bleomycin, Etoposide, Cisplatin
BIP	Bleomycin, Cisplatin, Ifosfamide, Mesna
BOMP	Bleomycin, Vincristine, Cisplatin, Mitomycin
CA	Cytarabine, Asparaginase
CABO	Cisplatin, Methotrexate, Bleomycin, Vincristine
CAF	Cyclophosphamide, Doxorubicin, Fluorouracil
CAL-G	Cyclophosphamide, Daunorubicin, Vincristine, Prednisone, Asparaginase
CAMP	Cyclophosphamide, Doxorubicin, Methotrexate, Procarbazine
CAP	Cyclophosphamide, Doxorubicin, Cisplatin
CaT	Carboplatin, Paclitaxel
CAV	Cyclophosphamide, Doxorubicin, Vincristine
CAVE ADD	CAV and Etoposide
CA-VP16	Cyclophosphamide, Doxorubicin, Etoposide
CC	Cyclophosphamide, Carboplatin
CDDP/VP-16	Cisplatin, Etoposide
CEF	Cyclophosphamide, Epirubicin, Fluorouracil
CEPP(B)	Cyclophosphamide, Etoposide, Prednisone, with or without/ Bleomycin
CEV	Cyclophosphamide, Etoposide, Vincristine
CF	Cisplatin, Fluorouracil or Carboplatin Fluorouracil
CHAP	Cyclophosphamide or Cyclophosphamide, Altretamine,

Name	Therapeutic agents
	Doxorubicin, Cisplatin
ChlVPP	Chlorambucil, Vinblastine, Procarbazine, Prednisone
CHOP	Cyclophosphamide, Doxorubicin, Vincristine, Prednisone
CHOP-BLEO	Add Bleomycin to CHOP
CISCA	Cyclophosphamide, Doxorubicin, Cisplatin
CLD-BOMP	Bleomycin, Cisplatin, Vincristine, Mitomycin
CMF	Methotrexate, Fluorouracil, Cyclophosphamide
CMFP	Cyclophosphamide, Methotrexate, Fluorouracil, Prednisone
CMFVP	Cyclophosphamide, Methotrexate, Fluorouracil, Vincristine, Prednisone
CMV	Cisplatin, Methotrexate, Vinblastine
CNF	Cyclophosphamide, Mitoxantrone, Fluorouracil
CNOP	Cyclophosphamide, Mitoxantrone, Vincristine, Prednisone
COB	Cisplatin, Vincristine, Bleomycin
CODE	Cisplatin, Vincristine, Doxorubicin, Etoposide
COMLA	Cyclophosphamide, Vincristine, Methotrexate, Leucovorin, Cytarabine
COMP	Cyclophosphamide, Vincristine, Methotrexate, Prednisone
Cooper Regimen	Cyclophosphamide, Methotrexate, Fluorouracil, Vincristine, Prednisone
COP	Cyclophosphamide, Vincristine, Prednisone
COPE	Cyclophosphamide, Vincristine, Cisplatin, Etoposide
COPP	Cyclophosphamide, Vincristine, Procarbazine, Prednisone
CP(Chronic lymphocytic leukemia)	Chlorambucil, Prednisone
CP (Ovarian Cancer)	Cyclophosphamide, Cisplatin
CT	Cisplatin, Paclitaxel
CVD	Cisplatin, Vinblastine, Dacarbazine
CVI	Carboplatin, Etoposide, Ifosfamide, Mesna
CVP	Cyclophosphamide, Vincristine, Prednisome
CVPP	Lomustine, Procarbazine, Prednisone

Name	Therapeutic agents
CYVADIC	Cyclophosphamide, Vincristine, Doxorubicin, Dacarbazine
DA	Daunorubicin, Cytarabine
DAT	Daunorubicin, Cytarabine, Thioguanine
DAV	Daunorubicin, Cytarabine, Etoposide
DCT	Daunorubicin, Cytarabine, Thioguanine
DHAP	Cisplatin, Cytarabine, Dexamethasone
DI	Doxorubicin, Ifosfamide
DTIC/Tamoxifen	Dacarbazine, Tamoxifen
DVP	Daunorubicin, Vincristine, Prednisone
EAP	Etoposide, Doxorubicin, Cisplatin
EC	Etoposide, Carboplatin
EFP	Etoposie, Fluorouracil, Cisplatin
ELF	Etoposide, Leucovorin, Fluorouracil
EMA 86	Mitoxantrone, Etoposide, Cytarabine
EP	Etoposide, Cisplatin
EVA	Etoposide, Vinblastine
FAC	Fluorouracil, Doxorubicin, Cyclophosphamide
FAM	Fluorouracil, Doxorubicin, Mitomycin
FAMTX	Methotrexate, Leucovorin, Doxorubicin
FAP	Fluorouracil, Doxorubicin, Cisplatin
F-CL	Fluorouracil, Leucovorin
FEC	Fluorouracil, Cyclophosphamide, Epirubicin
FED	Fluorouracil, Etoposide, Cisplatin
FL	Flutamide, Leuprolide
FZ	Flutamide, Goserelin acetate implant
HDMTX	Methotrexate, Leucovorin
Hexa-CAF	Altretamine, Cyclophosphamide, Methotrexate, Fluorouracil
ICE-T	Ifosfamide, Carboplatin, Etoposide, Paclitaxel, Mesna
IDMTX/6-MP	Methotrexate, Mercaptopurine, Leucovorin

Name	Therapeutic agents
IE	Ifosfamide, Etoposie, Mesna
IfoVP	Ifosfamide, Etoposide, Mesna
IPA	Ifosfamide, Cisplatin, Doxorubicin
M-2	Vincristine, Carmustine, Cyclophosphamide, Prednisone, Melphalan
MAC-III	Methotrexate, Leucovorin, Dactinomycin, Cyclophosphamide
MACC	Methotrexate, Doxorubicin, Cyclophosphamide, Lomustine
MACOP-B	Methotrexate, Leucovorin, Doxorubicin, Cyclophosphamide, Vincristine, Bleomycin, Prednisone
MAID	Mesna, Doxorubicin, Ifosfamide, Dacarbazine
m-BACOD	Bleomycin, Doxorubicin, Cyclophosphamide, Vincristine, Dexamethasone, Methotrexate, Leucovorin
MBC	Methotrexate, Bleomycin, Cisplatin
MC	Mitoxantrone, Cytarabine
MF	Methotrexate, Fluorouracil, Leucovorin
MICE	Ifosfamide, Carboplatin, Etoposide, Mesna
MINE	Mesna, Ifosfamide, Mitoxantrone, Etoposide
mini-BEAM	Carmustine, Etoposide, Cytarabine, Melphalan
MOBP	Bleomycin, Vincristine, Cisplatin, Mitomycin
MOP	Mechlorethamine, Vincristine, Procarbazine
MOPP	Mechlorethamine, Vincristine, Procarbazine, Prednisone
MOPP/ABV	Mechlorethamine, Vincristine, Procarbazine, Prednisone, Doxorubicin, Bleomycin, Vinblastine
MP (multiple myeloma)	Melphalan, Prednisone
MP (prostate cancer)	Mitoxantrone, Prednisone
MTX/6-MO	Methotrexate, Mercaptopurine
MTX/6-MP/VP	Methotrexate, Mercaptopurine, Vincristine, Prednisone
MTX-CDDPAdr	Methotrexate, Leucovorin, Cisplatin, Doxorubicin
MV (breast cancer)	Mitomycin, Vinblastine

Name	Therapeutic agents
MV (acute myelocytic leukemia)	Mitoxantrone, Etoposide
M-VAC Methotrexate	Vinblastine, Doxorubicin, Cisplatin
MVP Mitomycin	Vinblastine, Cisplatin
MVPP	Mechlorethamine, Vinblastine, Procarbazine, Prednisone
NFL	Mitoxantrone, Fluorouracil, Leucovorin
NOVP	Mitoxantrone, Vinblastine, Vincristine
OPA	Vincristine, Prednisone, Doxorubicin
OPPA	Add Procarbazine to OPA.
PAC	Cisplatin, Doxorubicin
PAC-I	Cisplatin, Doxorubicin, Cyclophosphamide
PA-CI	Cisplatin, Doxorubicin
PC	Paclitaxel, Carboplatin or Paclitaxel, Cisplatin
PCV	Lomustine, Procarbazine, Vincristine
PE	Paclitaxel, Estramustine
PFL	Cisplatin, Fluorouracil, Leucovorin
POC	Prednisone, Vincristine, Lomustine
ProMACE	Prednisone, Methotrexate, Leucovorin, Doxorubicin, Cyclophosphamide, Etoposide
ProMACE/cytaBOM	Prednisone, Doxorubicin, Cyclophosphamide, Etoposide, Cytarabine, Bleomycin, Vincristine, Methotrexate, Leucovorin, Cotrimoxazole
PRoMACE/MOPP	Prednisone, Doxorubicin, Cyclophosphamide, Etoposide, Mechlorethamine, Vincristine, Procarbazine, Methotrexate, Leucovorin
Pt/VM	Cisplatin, Teniposide
PVA	Prednisone, Vincristine, Asparaginase
PVB	Cisplatin, Vinblastine, Bleomycin
PVDA	Prednisone, Vincristine, Daunorubicin, Asparaginase
SMF	Streptozocin, Mitomycin, Fluorouracil
TAD	Mechlorethamine, Doxorubicin, Vinblastine, Vincristine, Bleomycin, Etoposide, Prednisone

Name	Therapeutic agents
TCF	Paclitaxel, Cisplatin, Fluorouracil
TIP	Paclitaxel, Ifosfamide, Mesna, Cisplatin
TTT	Methotrexate, Cytarabine, Hydrocortisone
Topo/CTX	Cyclophosphamide, Topotecan, Mesna
VAB-6	Cyclophosphamide, Dactinomycin, Vinblastine, Cisplatin, Bleomycin
VAC	Vincristine, Dactinomycin, Cyclophosphamide
VACAdr	Vincristine, Cyclophosphamide, Doxorubicin, Dactinomycin, Vincristine
VAD	Vincristine, Doxorubicin, Dexamethasone
VATH	Vinblastine, Doxorubicin, Thiotepa, Flouxymesterone
VBAP	Vincristine, Carmustine, Doxorubicin, Prednisone
VBCMP	Vincristine, Carmustine, Melphalan, Cyclophosphamide, Prednisone
VC	Vinorelbine, Cisplatin
VCAP	Vincristine, Cyclophosphamide, Doxorubicin, Prednisone
VD	Vinorelbine, Doxorubicin
VelP	Vinblastine, Cisplatin, Ifosfamide, Mesna
VIP	Etoposide, Cisplatin, Ifosfamide, Mesna
VM	Mitomycin, Vinblastine
VMCP	Vincristine, Melphalan, Cyclophosphamide, Prednisone
VP	Etoposide, Cisplatin
V-TAD	Etoposide, Thioguanine, Daunorubicin, Cytarabine
5 + 2	Cytarabine, Daunorubicin, Mitoxantrone
7 + 3	Cytarabine with/, Daunorubicin or Idarubicin or Mitoxantrone
"8 in 1"	Methylprednisolone, Vincristine, Lomustine, Procarbazine, Hydroxyurea, Cisplatin, Cytarabine, Dacarbazine

In certain embodiments, the compounds of the invention may be conjointly administered with a chemotherapeutic agent selected from inhibitors of another

metabolic enzyme, such as a glucose transporter, hexokinase, pyruvate kinase M2, lactate dehydrogenase 1 or 2, pyruvate dehydrogenase kinase, fatty acid synthase, or glutaminase.

In some embodiments, the compounds of the invention may be conjointly administered with an immune-oncology therapeutic, such as an inhibitor of arginase, CTLA-4, indoleamine 2,3-dioxygenase, and PD-1/PD-L1. In certain embodiments, the immune-oncology agent is abagovomab, adecatumumab, afutuzumab, anatumomab mafenatox, apolizumab, blinatumomab, catumaxomab, durvalumab, epratuzumab, indoximod, inotuzumab ozogamicin, intelumumab, ipilimumab, isatuximab, lambrolizumab, nivolumab, ocaratuzumab, olatatumab, pembrolizumab, pidilizumab, ticilimumab, samalizumab, or tremelimumab.

In certain embodiments, the compounds of the invention may be conjointly administered with an immunomodulatory agent. Examples of immunomodulatory agents with which the compounds of the invention may be administered in a combination therapy include granulocyte colony-stimulating factor (G-CSF), interferons, imiquimod, IL-2, IL-7, IL-12, various chemokines, synthetic cytosine phosphate-guanosine (CpG) oligodeoxynucleotides, glucans, and synthetic small molecules such as apremilast, CC-122, CC-11006, CC-10015, lenalidomide, pomalidomide, and thalidomide. In certain embodiments, the immunomodulatory agent is a thalidomide analog, such as those disclosed in WO 1999/46258, WO 2008/033567, WO 2010/093434, WO 2010/093605, WO 2011/100380, and WO 2012/097116.

In certain embodiments, the compounds of the invention may be conjointly administered with an anticancer agent selected from an enzyme inhibitor (such as a kinase inhibitor), a mitotic inhibitor, a DNA-modifying agent, and a cytidine analog. Examples of anticancer agents with which the compounds of the invention may be administered in a combination therapy include microtubule assembly inhibitors, AKT inhibitors, mTOR inhibitors, MEK inhibitors, RTK inhibitors, ATM inhibitors, ATR inhibitors, PI3K inhibitors, EGFR inhibitors, B-Raf inhibitors, C-kit inhibitors, DNA cross-linking agents, DNA intercalating agents, and cytidine analogs. In certain embodiments, the anticancer agents include microtubule assembly inhibitors, AKT inhibitors, mTOR inhibitors, MEK inhibitors, RTK inhibitors, ATM inhibitors, ATR inhibitors, PI3K inhibitors, EGFR inhibitors, B-Raf inhibitors, C-kit inhibitors, DNA cross-linking agents, PARP inhibitors, DNA intercalating agents, or cytidine analogs.

In certain embodiments, the anticancer agent is vincristine, carboplatin, cisplatin, gemcitabine, MK2206, everolimus, trametinib, sunitinib, sorafenib, BEZ235, paclitaxel, docetaxel, erlotinib, selumetinib, sirolimus, trametinib, temsirolimus, pazopanib, or GSK1120212. In certain embodiments, the anticancer agent is vincristine, carboplatin, cisplatin, gemcitabine, MK2206, everolimus, trametinib, sunitinib, sorafenib, BEZ235, paclitaxel, docetaxel, erlotinib, selumetinib, sirolimus, trametinib, temsirolimus, pazopanib, olaparib, or GSK1120212.

In certain embodiments, the compounds of the invention are coadministered with one or more of lenalidomide, pomalidomide, and dexamethasone in the treatment of multiple myeloma. In certain embodiments, coadministration produces a synergistic effect.

The proliferation of cancer cells requires lipid synthesis. Normally, acetyl-coA used for lipid synthesis is formed from a mitochondrial pool of pyruvate that is derived from glycolysis. Yet under hypoxic conditions, such as those normally found in a tumor environment, the conversion of pyruvate to acetyl-coA within the mitochondria is downregulated. Recent studies from Metallo et al. (2011) and Mullen et al. (2011) revealed that under such hypoxic conditions, cells instead largely switch to using a pathway involving the reductive carboxylation of alpha-ketoglutarate to make acetyl-coA for lipid synthesis. The first step in this pathway involves converting glutamine to glutamate via glutaminase enzymes. Subsequently, glutamate is converting to alpha-ketoglutarate, and the resulting alpha-ketoglutarate is converted to isocitrate in a reductive carboxylation step mediated by the isocitrate dehydrogenase enzymes. A switch to this reductive carboxylation pathway also occurs in some renal carcinoma cell lines that contain either impaired mitochondria or an impaired signal for induction of the enzyme responsible for converting glycolytic pyruvate to acetyl-coA (Mullen et al 2011). A similar switch occurs in cells exposed to mitochondrial respiratory chain inhibitors such as metformin, rotenone, and antimycin (Mullen at al. 2011). Therefore, in some embodiments of this invention, we propose using combinations of mitochondrial respiratory chain inhibitors and glutaminase inhibitors to simultaneously increase cancer cells' dependence on glutaminase-dependent pathways for lipid synthesis while inhibiting those very pathways.

The increased dependence on glycolysis in tumor cells is likely because the hypoxic tumor environment impairs mitochondrial respiration. Furthermore,

depletion of glucose induces apoptosis in cells transformed with the MYC oncogene. These findings suggest that inhibiting glycolysis would have a therapeutic value in preventing cancer cell proliferation. There are currently many documented glycolytic inhibitors (Pelicano et al. 2006). However, as pointed out by Zhao et al. (2012), "available glycolytic inhibitors are generally not very potent, and high doses are required, which may cause high levels of systemic toxicity." Since cancer cells typically use both glucose and glutamine at higher levels than normal cells, impairing utilization of each of those metabolites will likely have a synergistic effect. Therefore, in some embodiments of this invention, we propose using combinations of glycolytic pathway inhibitors and glutaminase inhibitors. Such glycolytic inhibitors include 2-deoxyglucose, lonidamine, 3-bromopyruvate, imatinib, oxythiamine, rapamycin, and their pharmacological equivalents. Glycolysis can be inhibited indirectly by depleting NAD+ via DNA damage induced by DNA alkylating agents through a pathway activated by poly(ADP-ribose) polymerase (Zong et al. 2004). Therefore, in some embodiments of this invention, we propose using a combination of DNA alkylating agents and glutaminase inhibitors. Cancer cells use the pentose phosphate pathway along with the glycolytic pathway to create metabolic intermediates derived from glucose. Therefore, in some embodiments of this invention, we propose using a combination of pentose phosphate inhibitors such as 6-aminonicotinamide along with glutaminase inhibitors.

In certain embodiments, a compound of the invention may be conjointly administered with non-chemical methods of cancer treatment. In certain embodiments, a compound of the invention may be conjointly administered with radiation therapy. In certain embodiments, a compound of the invention may be conjointly administered with surgery, with thermoablation, with focused ultrasound therapy, with cryotherapy, or with any combination of these.

In certain embodiments, different compounds of the invention may be conjointly administered with one or more other compounds of the invention. Moreover, such combinations may be conjointly administered with other therapeutic agents, such as other agents suitable for the treatment of cancer, immunological or neurological diseases, such as the agents identified above. In certain embodiments, conjointly administering one or more additional chemotherapeutic agents with a compound of the invention provides a synergistic effect, such as shown in Figure 8.

In certain embodiments, conjointly administering one or more additional chemotherapeutics agents provides an additive effect.

In certain embodiments, the present invention provides a kit comprising: a) one or more single dosage forms of a compound of the invention; b) one or more single dosage forms of a chemotherapeutic agent as mentioned above; and c) instructions for the administration of the compound of the invention and the chemotherapeutic agent for the treatment of cancer, wherein the cancer is multiple myeloma.

The present invention provides a kit comprising:

a) a pharmaceutical formulation (e.g., one or more single dosage forms) comprising a compound of the invention; and

b) instructions for the administration of the pharmaceutical formulation, e.g., for treating or preventing cancer, such as multiple myeloma.

In certain embodiments, the kit further comprises instructions for the administration of the pharmaceutical formulation comprising a compound of the invention conjointly with a chemotherapeutic agent as mentioned above. In certain embodiments, the kit further comprises a second pharmaceutical formulation (e.g., as one or more single dosage forms) comprising a chemotherapeutic agent as mentioned above.

Protein amounts can be measured using antibodies. Antibodies suitable for use in the methods disclosed herein are commercially available, or can be prepared routinely. Methods for preparing and using antibodies in assays for proteins of interest are conventional, and are described in, for example, Green et al., Production of Polyclonal Antisera, in Immunochemical Protocols (Manson, ed.), (Humana Press 1992); Coligan et al., in Current Protocols in Immunology, Sec. 2.4.1 (1992); Kohler & Milstein (1975), Nature 256, 495; Coligan et al., sections 2.5.1-2.6.7; and Harlow et al., Antibodies: A Laboratory Manual, page 726 (Cold Spring Harbor Laboratory Pub. 1988).

Any of a variety of antibodies can be used in methods of the invention. Such antibodies include, for example, polyclonal, monoclonal (mAbs), recombinant, humanized or partially humanized, single chain, Fab, and fragments thereof. The antibodies can be of any isotype, e.g., IgM, various IgG isotypes such as IgG1, IgG2a, etc., and they can be from any animal species that produces antibodies, including

goat, rabbit, mouse, chicken or the like. The term "an antibody specific for" a protein means that the antibody recognizes a defined sequence of amino acids, or epitope, in the protein, and binds selectively to the protein and not generally to proteins unintended for binding to the antibody. The parameters required to achieve specific binding can be determined routinely, using conventional methods in the art.

In some embodiments of the invention, antibodies specific for pyruvate carboxylase are immobilized on a surface (e.g., are reactive elements on an array, such as a microarray, or are on another surface, such as used for surface plasmon resonance (SPR)-based technology, such as Biacore), and proteins in the sample are detected by virtue of their ability to bind specifically to the antibodies. Alternatively, proteins in the sample can be immobilized on a surface, and detected by virtue of their ability to bind specifically to the antibodies. Methods of preparing the surfaces and performing the analyses, including conditions effective for specific binding, are conventional and well-known in the art.

Among the many types of suitable immunoassays are immunohistochemical staining, ELISA, Western blot (immunoblot), immunoprecipitation, radioimmunoassay (RIA), fluorescence-activated cell sorting (FACS), etc. Assays used in methods of the invention can be based on colorimetric readouts, fluorescent readouts, mass spectroscopy, visual inspection, etc.

As mentioned above, expression levels of pyruvate carboxylase can be measured by measuring mRNA amounts. The amount of an mRNA encoding a pyruvate carboxylase can be measured using any suitable method. Examples of such methods include, for example, reverse transcriptase-polymerase chain reaction (RT-PCR), including real time PCR, microarray analysis, nanostring, Northern blot analysis, differential hybridization, and ribonuclease protection assay. Such methods are well-known in the art and are described in, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, current edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., and Ausubel et al., Current Protocols in Molecular Biology, John Wiley & sons, New York, N.Y.

In some embodiments of the invention, a histological sample is obtained from a subject (e.g., from a tumor biopsy), using any method known in the art, and include, but are not limited to, tissue section, needle biopsy, and the like. Frequently the sample will be a "clinical sample", which is a sample derived from a patient, including sections of tissues such as frozen sections or paraffin sections taken for

histological purposes. The sample can also be derived from supernatants (of cells) or the cells themselves from cell cultures, cells from tissue culture and other media. Protein or mRNA is then obtained brom the sample, and used to quantitate the amounts of pyruvate carboxylase.

The disclosure also provides kits for detecting whether a subject having a cancer is likely to be responsive to glutaminase inhibitors. The kit may include one or more agents for detecting the amount of expression of a protein of the invention [e.g., the amount of the protein, and/or the amount of a nucleic acid (e.g., an mRNA) encoding the protein]. The agents in the kit can encompass, for example, antibodies specific for the proteins, or probes specific for the mRNA that can be used to hybridize to the RNA (or to a cDNA generated from it) or to perform RT-PCR. The kit may also include additional agents suitable for detecting, measuring and/or quantitating the amount of protein or nucleic acid. Among other uses, kits of the invention can be used in experimental applications. A skilled worker will recognize components of kits suitable for carrying out a method of the invention.

Optionally, a kit of the invention may comprise instructions for performing the method. Optional elements of a kit of the invention include suitable buffers, containers, or packaging materials. The reagents of the kit may be in containers in which the reagents are stable, e.g., in lyophilized form or stabilized liquids. The reagents may also be in single use form, e.g., for the performance of an assay for a single subject.

Definitions

The term “acyl” is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)-, preferably alkylC(O)-.

The term “acylamino” is art-recognized and refers to an amino group substituted with an acyl group and may be represented, for example, by the formula hydrocarbylC(O)NH-.

The term “acyloxy” is art-recognized and refers to a group represented by the general formula hydrocarbylC(O)O-, preferably alkylC(O)O-.

The term “alkoxy” refers to an alkyl group, preferably a lower alkyl group, having an oxygen attached thereto. Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy and the like.

The term “alkoxyalkyl” refers to an alkyl group substituted with an alkoxy group and may be represented by the general formula alkyl-O-alkyl.

The term “alkenyl”, as used herein, refers to an aliphatic group containing at least one double bond and is intended to include both "unsubstituted alkenyls" and "substituted alkenyls", the latter of which refers to alkenyl moieties having substituents replacing a hydrogen on one or more carbons of the alkenyl group. Such substituents may occur on one or more carbons that are included or not included in one or more double bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed below, except where stability is prohibitive. For example, substitution of alkenyl groups by one or more alkyl, carbocyclyl, aryl, heterocyclyl, or heteroaryl groups is contemplated.

An “alkyl” group or “alkane” is a straight chained or branched non-aromatic hydrocarbon which is completely saturated. Typically, a straight chained or branched alkyl group has from 1 to about 20 carbon atoms, preferably from 1 to about 10 unless otherwise defined. Examples of straight chained and branched alkyl groups include methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, pentyl, hexyl, pentyl and octyl. A C_1-C_6 straight chained or branched alkyl group is also referred to as a "lower alkyl" group.

Moreover, the term "alkyl" (or "lower alkyl") as used throughout the specification, examples, and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents, if not otherwise specified, can include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include substituted and unsubstituted forms of amino, azido, imino, amido, phosphoryl (including phosphonate and phosphinate), sulfonyl (including sulfate, sulfonamido, sulfamoyl and sulfonate), and silyl groups, as well as ethers, alkylthios,

carbonyls (including ketones, aldehydes, carboxylates, and esters), -CF_3, -CN and the like. Exemplary substituted alkyls are described below. Cycloalkyls can be further substituted with alkyls, alkenyls, alkoxys, alkylthios, aminoalkyls, carbonyl-substituted alkyls, -CF_3, -CN, and the like.

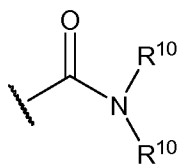
The term "C_{x-y}" when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups that contain from x to y carbons in the chain. For example, the term "C_{x-y}alkyl" refers to substituted or unsubstituted saturated hydrocarbon groups, including straight-chain alkyl and branched-chain alkyl groups that contain from x to y carbons in the chain, including haloalkyl groups such as trifluoromethyl and 2,2,2-tirfluoroethyl, etc. C_0 alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal. The terms "C_{2-y}alkenyl" and "C_{2-y}alkynyl" refer to substituted or unsubstituted unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

The term "alkylamino", as used herein, refers to an amino group substituted with at least one alkyl group.

The term "alkylthio", as used herein, refers to a thiol group substituted with an alkyl group and may be represented by the general formula alkylS-.

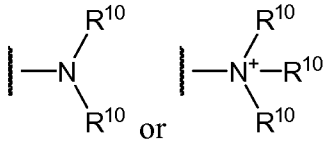
The term "alkynyl", as used herein, refers to an aliphatic group containing at least one triple bond and is intended to include both "unsubstituted alkynyls" and "substituted alkynyls", the latter of which refers to alkynyl moieties having substituents replacing a hydrogen on one or more carbons of the alkynyl group. Such substituents may occur on one or more carbons that are included or not included in one or more triple bonds. Moreover, such substituents include all those contemplated for alkyl groups, as discussed above, except where stability is prohibitive. For example, substitution of alkynyl groups by one or more alkyl, carbocyclyl, aryl, heterocyclyl, or heteroaryl groups is contemplated.

The term "amide", as used herein, refers to a group



wherein each R^{10} independently represent a hydrogen or hydrocarbyl group, or two R^{10} are taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines and salts thereof, e.g., a moiety that can be represented by



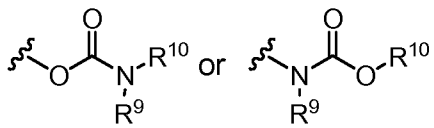
wherein each R^{10} independently represents a hydrogen or a hydrocarbyl group, or two R^{10} are taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term "aminoalkyl", as used herein, refers to an alkyl group substituted with an amino group.

The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group.

The term "aryl" as used herein include substituted or unsubstituted single-ring aromatic groups in which each atom of the ring is carbon. Preferably the ring is a 5- to 7-membered ring, more preferably a 6-membered ring. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Aryl groups include benzene, naphthalene, phenanthrene, phenol, aniline, and the like.

The term "carbamate" is art-recognized and refers to a group



wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl group, such as an alkyl group, or R^9 and R^{10} taken together with the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

The terms "carbocycle", and "carbocyclic", as used herein, refers to a saturated or unsaturated ring in which each atom of the ring is carbon. The term carbocycle includes both aromatic carbocycles and non-aromatic carbocycles. Non-

aromatic carbocycles include both cycloalkane rings, in which all carbon atoms are saturated, and cycloalkene rings, which contain at least one double bond. "Carbocycle" includes 5-7 membered monocyclic and 8-12 membered bicyclic rings. Each ring of a bicyclic carbocycle may be selected from saturated, unsaturated and aromatic rings. Carbocycle includes bicyclic molecules in which one, two or three or more atoms are shared between the two rings. The term "fused carbocycle" refers to a bicyclic carbocycle in which each of the rings shares two adjacent atoms with the other ring. Each ring of a fused carbocycle may be selected from saturated, unsaturated and aromatic rings. In an exemplary embodiment, an aromatic ring, e.g., phenyl, may be fused to a saturated or unsaturated ring, e.g., cyclohexane, cyclopentane, or cyclohexene. Any combination of saturated, unsaturated and aromatic bicyclic rings, as valence permits, is included in the definition of carbocyclic. Exemplary "carbocycles" include cyclopentane, cyclohexane, bicyclo[2.2.1]heptane, 1,5-cyclooctadiene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]oct-3-ene, naphthalene and adamantane. Exemplary fused carbocycles include decalin, naphthalene, 1,2,3,4-tetrahydronaphthalene, bicyclo[4.2.0]octane, 4,5,6,7-tetrahydro-1H-indene and bicyclo[4.1.0]hept-3-ene. "Carbocycles" may be susbstituted at any one or more positions capable of bearing a hydrogen atom.

A "cycloalkyl" group is a cyclic hydrocarbon which is completely saturated. "Cycloalkyl" includes monocyclic and bicyclic rings. Typically, a monocyclic cycloalkyl group has from 3 to about 10 carbon atoms, more typically 3 to 8 carbon atoms unless otherwise defined. The second ring of a bicyclic cycloalkyl may be selected from saturated, unsaturated and aromatic rings. Cycloalkyl includes bicyclic molecules in which one, two or three or more atoms are shared between the two rings. The term "fused cycloalkyl" refers to a bicyclic cycloalkyl in which each of the rings shares two adjacent atoms with the other ring. The second ring of a fused bicyclic cycloalkyl may be selected from saturated, unsaturated and aromatic rings. A "cycloalkenyl" group is a cyclic hydrocarbon containing one or more double bonds.

The term "carbocyclylalkyl", as used herein, refers to an alkyl group substituted with a carbocycle group.

The term "carbonate" is art-recognized and refers to a group $-OCO_2-R^{10}$, wherein R^{10} represents a hydrocarbyl group.

The term "carboxy", as used herein, refers to a group represented by the formula $-CO_2H$.

The term "ester", as used herein, refers to a group $-C(O)OR^{10}$ wherein R^{10} represents a hydrocarbyl group.

The term "ether", as used herein, refers to a hydrocarbyl group linked through an oxygen to another hydrocarbyl group. Accordingly, an ether substituent of a hydrocarbyl group may be hydrocarbyl-O-. Ethers may be either symmetrical or unsymmetrical. Examples of ethers include, but are not limited to, heterocycle-O-heterocycle and aryl-O-heterocycle. Ethers include "alkoxyalkyl" groups, which may be represented by the general formula alkyl-O-alkyl.

The terms "halo" and "halogen" as used herein means halogen and includes chloro, fluoro, bromo, and iodo.

The terms "hetaralkyl" and "heteroaralkyl", as used herein, refers to an alkyl group substituted with a hetaryl group.

The term "heteroalkyl", as used herein, refers to a saturated or unsaturated chain of carbon atoms and at least one heteroatom, wherein no two heteroatoms are adjacent.

The terms "heteroaryl" and "hetaryl" include substituted or unsubstituted aromatic single ring structures, preferably 5- to 7-membered rings, more preferably 5- to 6-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms "heteroaryl" and "hetaryl" also include polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heteroaromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heteroaryl groups include, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, pyridine, pyrazine, pyridazine, and pyrimidine, and the like.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, and sulfur.

The terms "heterocyclyl", "heterocycle", and "heterocyclic" refer to substituted or unsubstituted non-aromatic ring structures, preferably 3- to 10-membered rings, more preferably 3- to 7-membered rings, whose ring structures include at least one heteroatom, preferably one to four heteroatoms, more preferably one or two heteroatoms. The terms "heterocyclyl" and "heterocyclic" also include

polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings wherein at least one of the rings is heterocyclic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls. Heterocyclyl groups include, for example, piperidine, piperazine, pyrrolidine, morpholine, lactones, lactams, and the like.

The term "heterocyclylalkyl", as used herein, refers to an alkyl group substituted with a heterocycle group.

The term "hydrocarbyl", as used herein, refers to a group that is bonded through a carbon atom that does not have a =O or =S substituent, and typically has at least one carbon-hydrogen bond and a primarily carbon backbone, but may optionally include heteroatoms. Thus, groups like methyl, ethoxyethyl, 2-pyridyl, and trifluoromethyl are considered to be hydrocarbyl for the purposes of this application, but substituents such as acetyl (which has a =O substituent on the linking carbon) and ethoxy (which is linked through oxygen, not carbon) are not. Hydrocarbyl groups include, but are not limited to aryl, heteroaryl, carbocycle, heterocyclyl, alkyl, alkenyl, alkynyl, and combinations thereof.

The term "hydroxyalkyl", as used herein, refers to an alkyl group substituted with a hydroxy group.

The term "lower" when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups where there are ten or fewer non-hydrogen atoms in the substituent, preferably six or fewer. A "lower alkyl", for example, refers to an alkyl group that contains ten or fewer carbon atoms, preferably six or fewer. In certain embodiments, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy substituents defined herein are respectively lower acyl, lower acyloxy, lower alkyl, lower alkenyl, lower alkynyl, or lower alkoxy, whether they appear alone or in combination with other substituents, such as in the recitations hydroxyalkyl and aralkyl (in which case, for example, the atoms within the aryl group are not counted when counting the carbon atoms in the alkyl substituent).

The terms "polycyclyl", "polycycle", and "polycyclic" refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls, heteroaryls, and/or heterocyclyls) in which two or more atoms are common to two adjoining rings, e.g., the rings are "fused rings". Each of the rings of the polycycle can be substituted or

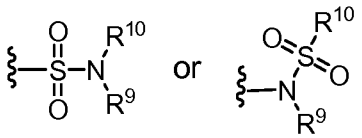
unsubstituted. In certain embodiments, each ring of the polycycle contains from 3 to 10 atoms in the ring, preferably from 5 to 7.

The term "silyl" refers to a silicon moiety with three hydrocarbyl moieties attached thereto.

The term "substituted" refers to moieties having substituents replacing a hydrogen on one or more carbons of the backbone. It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and non-aromatic substituents of organic compounds. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. Substituents can include any substituents described herein, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphate, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that substituents can themselves be substituted, if appropriate. Unless specifically stated as "unsubstituted," references to chemical moieties herein are understood to include substituted variants. For example, reference to an "aryl" group or moiety implicitly includes both substituted and unsubstituted variants.

The term "sulfate" is art-recognized and refers to the group $-OSO_3H$, or a pharmaceutically acceptable salt thereof.

The term "sulfonamide" is art-recognized and refers to the group represented by the general formulae



wherein R^9 and R^{10} independently represents hydrogen or hydrocarbyl, such as alkyl, or R^9 and R^{10} taken together with the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

The term "sulfoxide" is art-recognized and refers to the group -S(O)-R^{10}, wherein R^{10} represents a hydrocarbyl.

The term "sulfonate" is art-recognized and refers to the group SO_3H, or a pharmaceutically acceptable salt thereof.

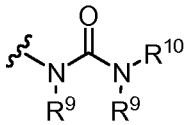
The term "sulfone" is art-recognized and refers to the group -$S(O)_2$-R^{10}, wherein R^{10} represents a hydrocarbyl.

The term "thioalkyl", as used herein, refers to an alkyl group substituted with a thiol group.

The term "thioester", as used herein, refers to a group -C(O)SR^{10} or -SC(O)R^{10} wherein R^{10} represents a hydrocarbyl.

The term "thioether", as used herein, is equivalent to an ether, wherein the oxygen is replaced with a sulfur.

The term "urea" is art-recognized and may be represented by the general formula



wherein R^9 and R^{10} independently represent hydrogen or a hydrocarbyl, such as alkyl, or either occurrence of R^9 taken together with R^{10} and the intervening atom(s) complete a heterocycle having from 4 to 8 atoms in the ring structure.

"Protecting group" refers to a group of atoms that, when attached to a reactive functional group in a molecule, mask, reduce or prevent the reactivity of the functional group. Typically, a protecting group may be selectively removed as desired during the course of a synthesis. Examples of protecting groups can be found in Greene and Wuts, *Protective Groups in Organic Chemistry,* 3rd Ed., 1999, John Wiley & Sons, NY and Harrison et al., *Compendium of Synthetic Organic Methods*, Vols. 1-8, 1971-1996, John Wiley & Sons, NY. Representative nitrogen protecting groups include, but are not limited to, formyl, acetyl, trifluoroacetyl, benzyl,

benzyloxycarbonyl ("CBZ"), tert-butoxycarbonyl ("Boc"), trimethylsilyl ("TMS"), 2-trimethylsilyl-ethanesulfonyl ("TES"), trityl and substituted trityl groups, allyloxycarbonyl, 9-fluorenylmethyloxycarbonyl ("FMOC"), nitro-veratryloxycarbonyl ("NVOC") and the like. Representative hydroxylprotecting groups include, but are not limited to, those where the hydroxyl group is either acylated (esterified) or alkylated such as benzyl and trityl ethers, as well as alkyl ethers, tetrahydropyranyl ethers, trialkylsilyl ethers (e.g., TMS or TIPS groups), glycol ethers, such as ethylene glycol and propylene glycol derivatives and allyl ethers.

The term "healthcare providers" refers to individuals or organizations that provide healthcare services to a person, community, etc. Examples of "healthcare providers" include doctors, hospitals, continuing care retirement communities, skilled nursing facilities, subacute care facilities, clinics, multispecialty clinics, freestanding ambulatory centers, home health agencies, and HMO's.

As used herein, a therapeutic that "prevents" a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample.

The term "treating" includes prophylactic and/or therapeutic treatments. The term "prophylactic or therapeutic" treatment is art-recognized and includes administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) then the treatment is prophylactic (i.e., it protects the host against developing the unwanted condition), whereas if it is administered after manifestation of the unwanted condition, the treatment is therapeutic, (i.e., it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof).

The term "prodrug" is intended to encompass compounds which, under physiologic conditions, are converted into the therapeutically active agents of the present invention (e.g., a compound of formula I). A common method for making a prodrug is to include one or more selected moieties which are hydrolyzed under physiologic conditions to reveal the desired molecule. In other embodiments, the prodrug is converted by an enzymatic activity of the host animal. For example, esters

or carbonates (e.g., esters or carbonates of alcohols or carboxylic acids) are preferred prodrugs of the present invention. In certain embodiments, some or all of the compounds of formula I in a formulation represented above can be replaced with the corresponding suitable prodrug, e.g., wherein a hydroxyl in the parent compound is presented as an ester or a carbonate or carboxylic acid present in the parent compound is presented as an ester.

Pharmaceutical Compositions

The compositions and methods of the present invention may be utilized to treat an individual in need thereof. In certain embodiments, the individual is a mammal such as a human, or a non-human mammal. When administered to an animal, such as a human, the composition or the compound is preferably administered as a pharmaceutical composition comprising, for example, a compound of the invention and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well known in the art and include, for example, aqueous solutions such as water or physiologically buffered saline or other solvents or vehicles such as glycols, glycerol, oils such as olive oil, or injectable organic esters. In a preferred embodiment, when such pharmaceutical compositions are for human administration, particularly for invasive routes of administration (i.e., routes, such as injection or implantation, that circumvent transport or diffusion through an epithelial barrier), the aqueous solution is pyrogen-free, or substantially pyrogen-free. The excipients can be chosen, for example, to effect delayed release of an agent or to selectively target one or more cells, tissues or organs. The pharmaceutical composition can be in dosage unit form such as tablet, capsule (including sprinkle capsule and gelatin capsule), granule, lyophile for reconstitution, powder, solution, syrup, suppository, injection or the like. The composition can also be present in a transdermal delivery system, e.g., a skin patch. The composition can also be present in a solution suitable for topical administration, such as an eye drop.

A pharmaceutically acceptable carrier can contain physiologically acceptable agents that act, for example, to stabilize, increase solubility or to increase the absorption of a compound such as a compound of the invention. Such physiologically acceptable agents include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients. The choice of a pharmaceutically acceptable carrier, including a physiologically acceptable agent,

depends, for example, on the route of administration of the composition. The preparation or pharmaceutical composition can be a selfemulsifying drug delivery system or a selfmicroemulsifying drug delivery system. The pharmaceutical composition (preparation) also can be a liposome or other polymer matrix, which can have incorporated therein, for example, a compound of the invention. Liposomes, for example, which comprise phospholipids or other lipids, are nontoxic, physiologically acceptable and metabolizable carriers that are relatively simple to make and administer.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

A pharmaceutical composition (preparation) can be administered to a subject by any of a number of routes of administration including, for example, orally (for example, drenches as in aqueous or non-aqueous solutions or suspensions, tablets, capsules (including sprinkle capsules and gelatin capsules), boluses, powders,

granules, pastes for application to the tongue); absorption through the oral mucosa (e.g., sublingually); anally, rectally or vaginally (for example, as a pessary, cream or foam); parenterally (including intramuscularly, intravenously, subcutaneously or intrathecally as, for example, a sterile solution or suspension); nasally; intraperitoneally; subcutaneously; transdermally (for example as a patch applied to the skin); and topically (for example, as a cream, ointment or spray applied to the skin, or as an eye drop). The compound may also be formulated for inhalation. In certain embodiments, a compound may be simply dissolved or suspended in sterile water. Details of appropriate routes of administration and compositions suitable for same can be found in, for example, U.S. Pat. Nos. 6,110,973, 5,731,000, 5,541,231, 5,427,798, 5,358,970 and 4,172,896, as well as in patents cited therein.

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient that can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 1 percent to about ninety-nine percent of active ingredient, preferably from about 5 percent to about 70 percent, most preferably from about 10 percent to about 30 percent.

Methods of preparing these formulations or compositions include the step of bringing into association an active compound, such as a compound of the invention, with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules (including sprinkle capsules and gelatin capsules), cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), lyophile, powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount

of a compound of the present invention as an active ingredient. Compositions or compounds may also be administered as a bolus, electuary or paste.

To prepare solid dosage forms for oral administration (capsules (including sprinkle capsules and gelatin capsules), tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; (10) complexing agents, such as, modified and unmodified cyclodextrins; and (11) coloring agents. In the case of capsules (including sprinkle capsules and gelatin capsules), tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions, such as dragees, capsules (including sprinkle capsules and gelatin capsules), pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also 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, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions that can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions that can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms useful for oral administration include pharmaceutically acceptable emulsions, lyophiles for reconstitution, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, cyclodextrins and derivatives thereof, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions for rectal, vaginal, or urethral administration may be presented as a suppository, which may be prepared by mixing one or more active compounds with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at

body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations of the pharmaceutical compositions for administration to the mouth may be presented as a mouthwash, or an oral spray, or an oral ointment.

Alternatively or additionally, compositions can be formulated for delivery via a catheter, stent, wire, or other intraluminal device. Delivery via such devices may be especially useful for delivery to the bladder, urethra, ureter, rectum, or intestine.

Formulations which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants that may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to an active compound, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the active compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention. Exemplary ophthalmic formulations are described in U.S. Publication Nos. 2005/0080056, 2005/0059744, 2005/0031697 and 2005/004074 and U.S. Patent No. 6,583,124, the contents of which

are incorporated herein by reference. If desired, liquid ophthalmic formulations have properties similar to that of lacrimal fluids, aqueous humor or vitreous humor or are compatable with such fluids. A preferred route of administration is local administration (*e.g.*, topical administration, such as eye drops, or administration via an implant).

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

Pharmaceutical compositions suitable for parenteral administration comprise one or more active compounds in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents that delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution, which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsulated matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions that are compatible with body tissue.

For use in the methods of this invention, active compounds can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

Methods of introduction may also be provided by rechargeable or biodegradable devices. Various slow release polymeric devices have been developed and tested *in vivo* in recent years for the controlled delivery of drugs, including proteinacious biopharmaceuticals. A variety of biocompatible polymers (including hydrogels), including both biodegradable and non-degradable polymers, can be used to form an implant for the sustained release of a compound at a particular target site.

Actual dosage levels of the active ingredients in the pharmaceutical compositions may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular compound or combination of compounds employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound(s) being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound(s) employed, the age, sex, weight, condition, general health and

prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the therapeutically effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the pharmaceutical composition or compound at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. By "therapeutically effective amount" is meant the concentration of a compound that is sufficient to elicit the desired therapeutic effect. It is generally understood that the effective amount of the compound will vary according to the weight, sex, age, and medical history of the subject. Other factors which influence the effective amount may include, but are not limited to, the severity of the patient's condition, the disorder being treated, the stability of the compound, and, if desired, another type of therapeutic agent being administered with the compound of the invention. A larger total dose can be delivered by multiple administrations of the agent. Methods to determine efficacy and dosage are known to those skilled in the art (Isselbacher *et al*. (1996) Harrison's Principles of Internal Medicine 13 ed., 1814-1882, herein incorporated by reference).

In general, a suitable daily dose of an active compound used in the compositions and methods of the invention will be that amount of the compound that is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above.

If desired, the effective daily dose of the active compound may be administered as one, two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. In certain embodiments of the present invention, the active compound may be administered two or three times daily. In preferred embodiments, the active compound will be administered once daily.

The patient receiving this treatment is any animal in need, including primates, in particular humans, and other mammals such as equines, cattle, swine and sheep; and poultry and pets in general.

In certain embodiments, compounds of the invention may be used alone or conjointly administered with another type of therapeutic agent. As used herein, the phrase "conjoint administration" refers to any form of administration of two or more

different therapeutic compounds such that the second compound is administered while the previously administered therapeutic compound is still effective in the body (*e.g.*, the two compounds are simultaneously effective in the patient, which may include synergistic effects of the two compounds). For example, the different therapeutic compounds can be administered either in the same formulation or in a separate formulation, either concomitantly or sequentially. In certain embodiments, the different therapeutic compounds can be administered within one hour, 12 hours, 24 hours, 36 hours, 48 hours, 72 hours, or a week of one another. Thus, an individual who receives such treatment can benefit from a combined effect of different therapeutic compounds.

In certain embodiments, conjoint administration of compounds of the invention with one or more additional therapeutic agent(s) (e.g., one or more additional chemotherapeutic agent(s)) provides improved efficacy relative to each individual administration of the compound of the invention (e.g., compound of formula I or Ia) or the one or more additional therapeutic agent(s). In certain such embodiments, the conjoint administration provides an additive effect, wherein an additive effect refers to the sum of each of the effects of individual administration of the compound of the invention and the one or more additional therapeutic agent(s).

This invention includes the use of pharmaceutically acceptable salts of compounds of the invention in the compositions and methods of the present invention. In certain embodiments, contemplated salts of the invention include, but are not limited to, alkyl, dialkyl, trialkyl or tetra-alkyl ammonium salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, L-arginine, benenthamine, benzathine, betaine, calcium hydroxide, choline, deanol, diethanolamine, diethylamine, 2-(diethylamino)ethanol, ethanolamine, ethylenediamine, N-methylglucamine, hydrabamine, 1H-imidazole, lithium, L-lysine, magnesium, 4-(2-hydroxyethyl)morpholine, piperazine, potassium, 1-(2-hydroxyethyl)pyrrolidine, sodium, triethanolamine, tromethamine, and zinc salts. In certain embodiments, contemplated salts of the invention include, but are not limited to, Na, Ca, K, Mg, Zn or other metal salts.

The pharmaceutically acceptable acid addition salts can also exist as various solvates, such as with water, methanol, ethanol, dimethylformamide, and the like. Mixtures of such solvates can also be prepared. The source of such solvate can be from the solvent of crystallization, inherent in the solvent of preparation or

crystallization, or adventitious to such solvent.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: (1) water-soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal-chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

In certain embodiments, the invention relates to a method for conducting a pharmaceutical business, by manufacturing a formulation of a compound of the invention, or a kit as described herein, and marketing to healthcare providers the benefits of using the formulation or kit for treating or preventing any of the diseases or conditions as described herein.

In certain embodiments, the invention relates to a method for conducting a pharmaceutical business, by providing a distribution network for selling a formulation of a compound of the invention, or kit as described herein, and providing instruction material to patients or physicians for using the formulation for treating or preventing any of the diseases or conditions as described herein.

In certain embodiments, the invention comprises a method for conducting a pharmaceutical business, by determining an appropriate formulation and dosage of a compound of the invention for treating or preventing any of the diseases or conditions as described herein, conducting therapeutic profiling of identified formulations for efficacy and toxicity in animals, and providing a distribution network for selling an identified preparation as having an acceptable therapeutic profile. In certain embodiments, the method further includes providing a sales group for marketing the preparation to healthcare providers.

In certain embodiments, the invention relates to a method for conducting a pharmaceutical business by determining an appropriate formulation and dosage of a compound of the invention for treating or preventing any of the disease or conditions

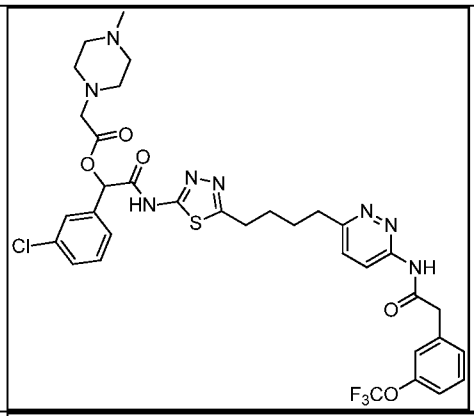
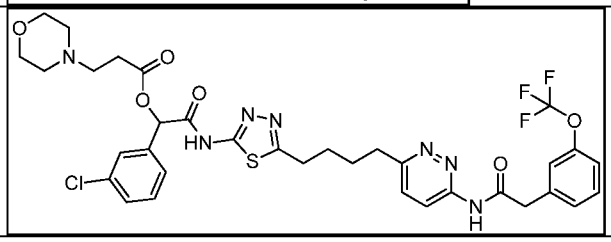
as described herein, and licensing, to a third party, the rights for further development and sale of the formulation.

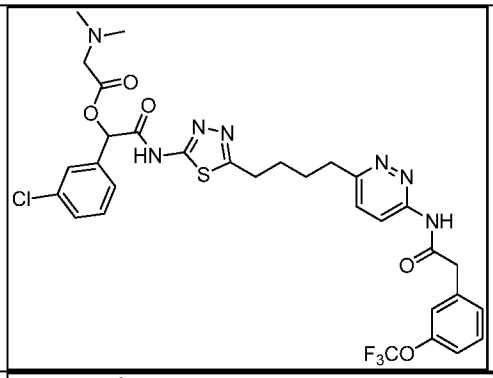
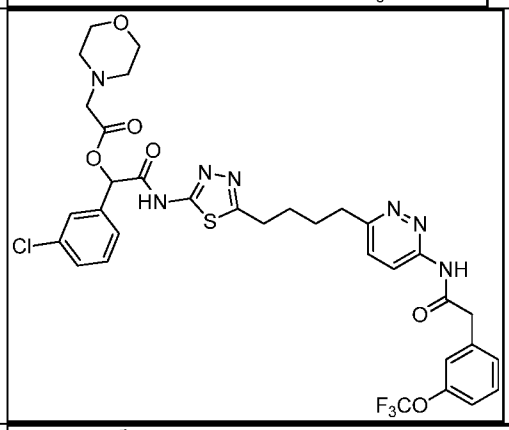
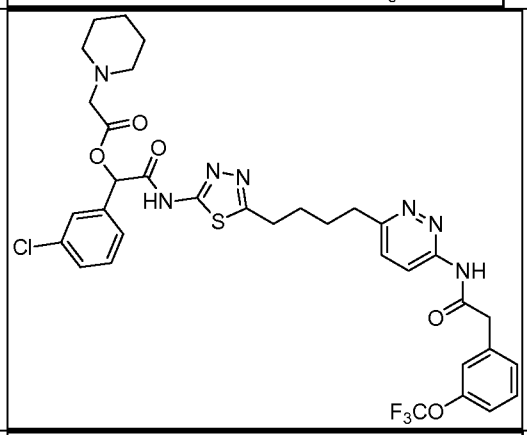
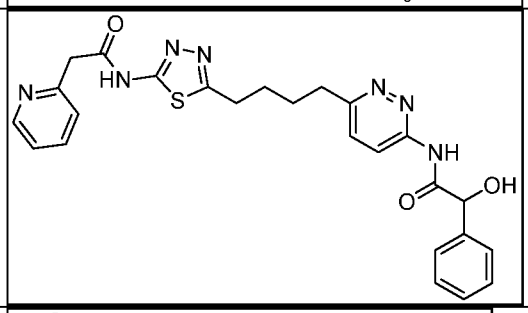
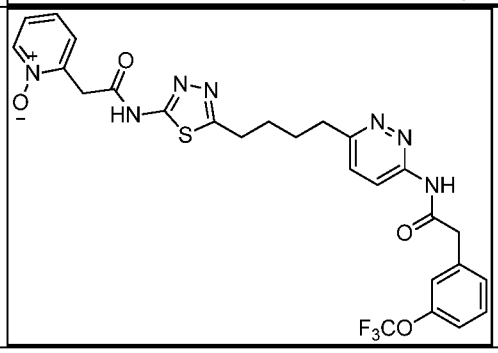
Examples

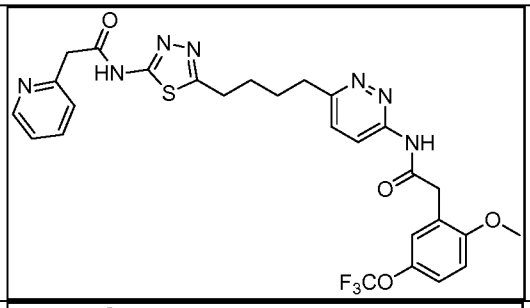
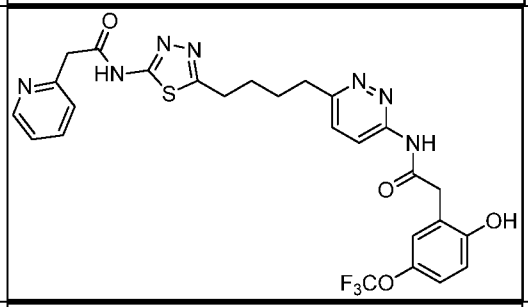
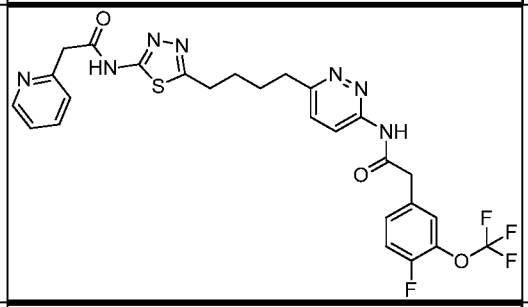
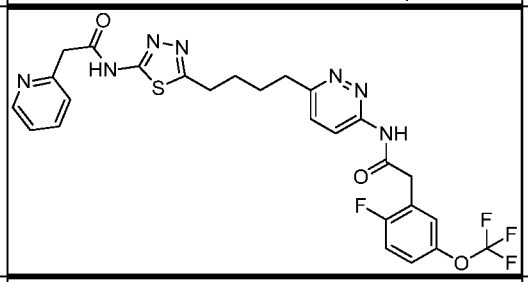
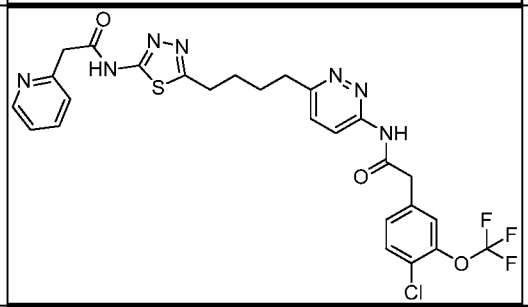
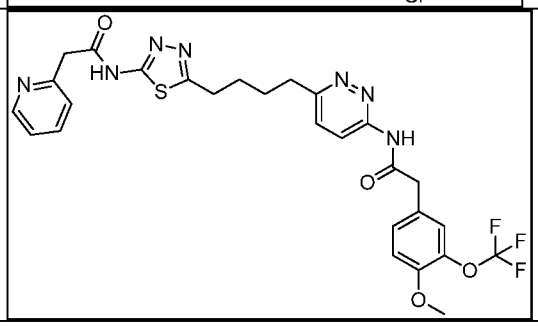
The synthesis of exemplary compounds of the invention is described in U.S. Patent No. 8,604,016, which is incorporated herein by reference. Also described in U.S. Patent No. 8,604,016 are protocols for various assays including recombinant enzyme assays and assays surveying cell proliferation, solubility, and Caco-2 permeability using the compounds of the invention. Certain data are found in Table 3, below.

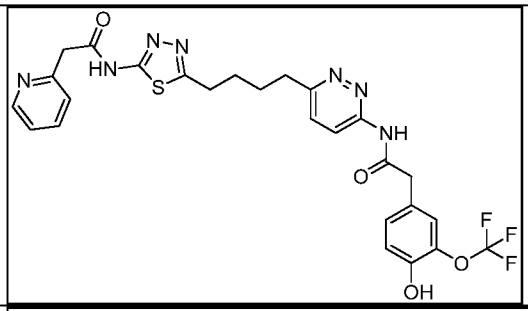
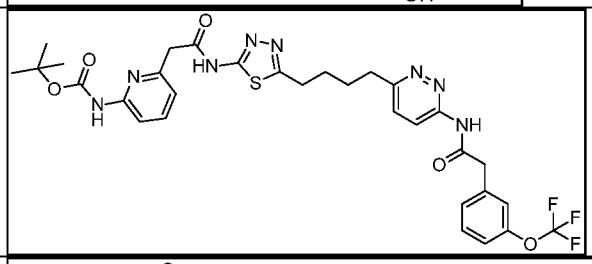
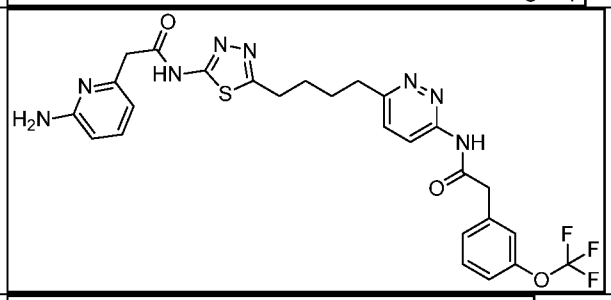
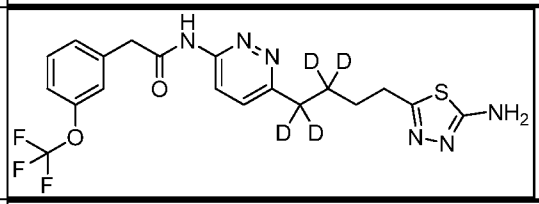
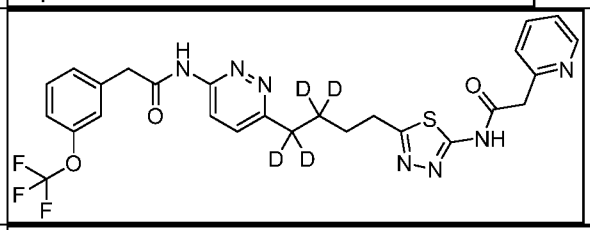
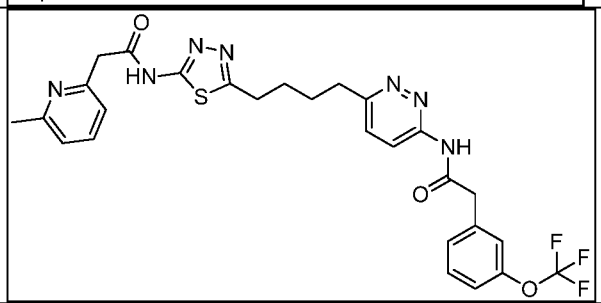
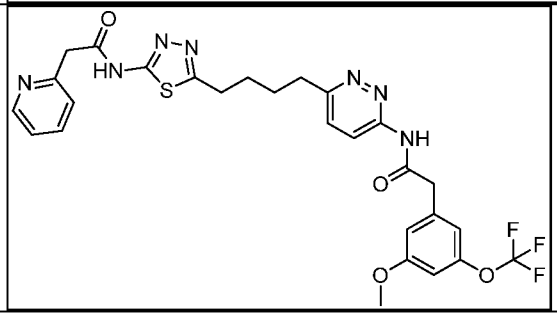
IC50 is a quantitative measure indicating how much compound is needed to inhibit a given biological activity by half.

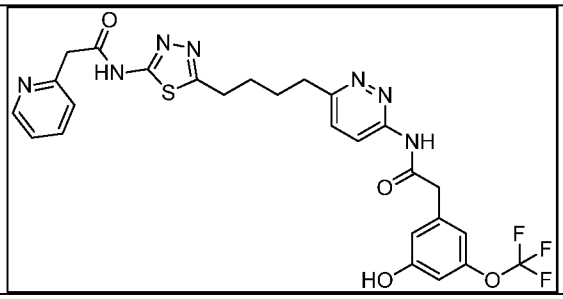
Table 3:

Cmpd ID	Structure	Modified GAC Delta N2 IC50 60 min preinc (μM)	GAC Delta N2 IC50 60 min preinc (μM)	GAC Delta N2 IC50 no preinc (μM)	Cell prolif P493 72h IC50 (μM)
710					
711					

712						
713						
714						
715			0.19			0.39
716						0.18

717			0.034			0.019
718			0.026			0.015
719			0.033			0.01
720			0.020			0.92
721			0.016			0.022
722			0.024			0.016

723		0.042			0.02
724		0.14			0.034
725		0.050			0.15
726		0.54			0.61
727		0.023			0.012
728		0.012			0.018
729		0.016			0.026

730			0.013		0.025

Further experimental data pertaining to use of the compounds of the invention in anti-proliferation studies, metabolite studies, and treatment of cancer xenografts can be found at Examples 5-16 of U.S. Patent Application Publication No. 2015/0004134, which is hereby incorporated by reference.

Example 5: Multiple Myeloma xenograft study.

Female CB.17 SCID mice (n=20) age 8-12 weeks were implanted subcutaneously with 1 x 10^7 RPMI-8226 myeloma cells per mouse mixed 1:1 with Matrigel. Mice were randomized into the following two groups of n=10 mice/group: 1) Vehicle control (25% Hydroxypropyl-β-cyclodextrin) and 2) Compound 670 dosed at orally at 200 mg/kg (formulated at 20 mg/mL in 25% HP-β-CD). For both groups, dosing was initiated when tumors reached a volume of 100-150mm^3 and continued orally BID for 28 days. Tumors were measured with calipers two times per week and tumor volume calculated using the formula tumor volume (mm^3) = (a x b^2/2) where 'b' is the smallest diameter and 'a' is the largest perpendicular diameter. **P-value < 0.01 (Two-sided T-test). Results are shown in Figure 17.

Example 6: Treatment of Multiple Myeloma cells with a combination of drugs.

As shown in Figure 18, MM1S cells (panels A & B) and RPMI-8226 cells (panels C & D) were treated with a dose titration of either compound 670, pomalidomide or a mixture thereof (panels A & C) or compound 670, dexamethsone or a mixture thereof (panels B & D) for 72 hours in growth media. At the end of the incubation, cell viability was measured using Cell Titer Glo as per manufacturer's protocol (Promega, Madison, WI). Measured values for compound-treated cells were normalized to DMSO-treated cells and data is reported as a cell survival ratio with a value of 1 (one) corresponding to maximum cell survival and a value of 0 (zero) corresponding to no cell survival. Cell survival ratios for all compound treatments are represented as bar graphs. Combination indices were calculated using the Calcusyn

program (biosoft.com) and reported for individual mixtures of compound 670 and pomalidomide [POM] (panels A &C) and individual mixtures of compound 670 and dexamethasone [DEX] (panels B & D). Compound mixtures that produced a synergistic anti-tumor activity are highlighted.

Example 7: SDS-PAGE and Western Blotting

Figure 10 shows a western blot for pyruvate carboxylase in multiple myeloma cell lines. Total cell lysates are prepared from cell pellets by sonication and protein concentration is quantitated using standard methods. Lysate proteins are denatured by boiling in SDS-sample buffer and resolved (10 μg/lane) by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins are then transferred to nitrocellulose and probed with antibodies recognizing pyruvate carboxylase or beta-actin followed by HRP-coupled secondary antibodies. Bands are revealed by chemiluminescence. Image J software (NIH) is used to quantify the pyruvate carboxylase band intensity using methods recommended by the software developer.

Example 8: Cell viability assay

Cells were treated with a dose range of CB-839 for 72 h at 37°C with 5% CO_2. Anti-proliferative effects were determined by measuring ATP levels using Cell Titer Glo reagent (Promega) following the manufactures' instructions. The Cell Titer Glo signal was measured on the day of compound addition and compared to the measurements taken at 72 h to determine effects on cell growth and survival during the assay period. Inhibition curves were fitted (GraphPad Prism) to a four-parameter dose response equation of the form: % activity = Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope)). Results are shown in Figure 11.

Whole Transcriptome Sequencing (RNA-Seq)

Whole transcriptome sequencing on cell pellets is performed by Expression Analysis (Durham, NC). Normalized expression values are determined using the RSEM (RNA-Seq by Expectation Maximization) software package. Normalized expression level is shown in Figure 12.

Incorporation by Reference

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control. The compounds, synthetic methods, and experimental protocols and results of U.S. Patent Number 8,604,016, filed November 19, 2012 and issued December 10, 2013, are hereby incorporated by reference.

Equivalents

While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive. Many variations of the invention will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

Claims:

1. A method for treating multiple myeloma in a patient, the method comprising:

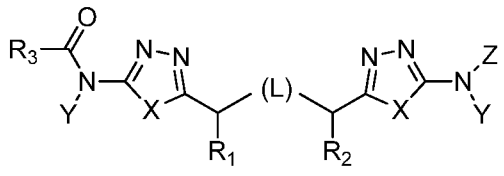
a) determining a level of pyruvate carboxylase in a patient sample comprising multiple myeloma cancer cells;

b) comparing the level of pyruvate carboxylase in the patient sample to a reference standard; and

c) if the level of pyruvate carboxylase in the patient sample is lower than the reference standard, then administering to the patient an effective amount of a glutaminase inhibitor.

2. A method of identifying a multiple myeloma patient that may benefit from treatment with a glutaminase inhibitor, comprising determining the level of pyruvate carboxylase in multiple myeloma cancer cells of the patient compared to a reference standard, wherein a lower level in the cancer cells of the patient as compared to the standard indicates that the patient may benefit from treatment with a glutaminase inhibitor.

3. The method of claim 1 or claim 2, wherein the glutaminase inhibitor is a compound of formula I,



(I),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or  , wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X, independently for each occurrence, represents S, O or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy;

R_3, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H.

4. The method of claim 3, wherein L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2.

5. The method of claim 3, wherein L represents CH_2CH_2.

6. The method of any one of claims 3-5, wherein Y represents H.

7. The method of any one of claims 3-6, wherein X, independently for each occurrence, represents S or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl.

8. The method of any one of claims 3-7, wherein Z represents $R_3(CO)$.

9. The method of claim 8, wherein each occurrence of R_3 is not identical.

10. The method of any one of claims 3-9, wherein R_1 and R_2 each represent H.

11. The method of any one of claims 3-10, wherein R_3, independently for each occurrence, represents substituted or unsubstituted arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl.

12. The method of any one of claims 3-11, wherein R_3, independently for each occurrence, represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl, heteroaryl or heteroaralkyl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl.

13. The method of claim 12, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl, or heteroaryl.

14. The method of claim 12 or 13, wherein R_{10} represents hydroxy, hydroxyalkyl, or alkoxy.

15. The method of claim 3, wherein L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent

H, and R_3, independently for each occurrence, represents substituted or unsubstituted arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl.

16. The method of claim 15, wherein each occurrence of R_3 is identical.

17. The method of claim 3, wherein L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and R_3, independently for each occurrence, represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl, heteroaryl or heteroaralkyl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl.

18. The method of claim 17, wherein L represents CH_2CH_2.

19. The method of claim 17 or 18, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl or heteroaryl.

20. The method of claim 19, wherein R_8 represents substituted or unsubstituted aryl.

21. The method of any of claims 17-20, wherein R_{10} represents hydroxy, hydroxyalkyl or alkoxy.

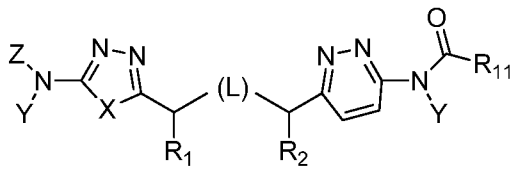
22. The method of claim 21, wherein R_{10} represents hydroxyalkyl.

23. The method of any one of claims 17-22, wherein each occurrence of R_3 is identical.

24. The method of claim 3, wherein L represents CH_2CH_2, Y represents H, X, independently for each occurrence, represents S or CH=CH, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and R_3, independently for each occurrence, represents arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl.

25. The method of claim 24, wherein each occurrence of R_3 is identical.

26. The method of claim 1 or claim 2, wherein the glutaminase inhibitor is a compound of formula Ia,



(Ia),

or a pharmaceutically acceptable salt thereof, wherein:

L represents CH_2SCH_2, CH_2CH_2, $CH_2CH_2CH_2$, CH_2, CH_2S, SCH_2, CH_2NHCH_2, CH=CH, or  , preferably CH_2CH_2, wherein any hydrogen atom of a CH or CH_2 unit may be replaced by alkyl or alkoxy, any hydrogen of an NH unit may be replaced by alkyl, and any hydrogen atom of a CH_2 unit of CH_2CH_2, $CH_2CH_2CH_2$ or CH_2 may be replaced by hydroxy;

X represents S, O or CH=CH, preferably S or CH=CH, wherein any hydrogen atom of a CH unit may be replaced by alkyl;

Y, independently for each occurrence, represents H or $CH_2O(CO)R_7$;

R_7, independently for each occurrence, represents H or substituted or unsubstituted alkyl, alkoxy, aminoalkyl, alkylaminoalkyl, heterocyclylalkyl, arylalkyl, or heterocyclylalkoxy;

Z represents H or $R_3(CO)$;

R_1 and R_2 each independently represent H, alkyl, alkoxy or hydroxy, preferably H;

R_3 represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, heteroaryloxyalkyl or $C(R_8)(R_9)(R_{10})$, $N(R_4)(R_5)$ or OR_6, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_4 and R_5 each independently represent H or substituted or unsubstituted alkyl, hydroxyalkyl, acyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_6, independently for each occurrence, represents substituted or unsubstituted alkyl, hydroxyalkyl, aminoalkyl, acylaminoalkyl, alkenyl, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$; and

R_8, R_9 and R_{10} each independently represent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or R_8 and R_9 together with the carbon to which they are attached, form a carbocyclic or heterocyclic ring system, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein at least two of R_8, R_9 and R_{10} are not H;

R_{11} represents substituted or unsubstituted aryl, arylalkyl, aryloxy, aryloxyalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, or $C(R_{12})(R_{13})(R_{14})$, $N(R_4)(R_{14})$ or OR_{14}, wherein any free hydroxyl group may be acylated to form $C(O)R_7$;

R_{12} and R_{13} each independently respresent H or substituted or unsubstituted alkyl, hydroxy, hydroxyalkyl, amino, acylamino, aminoalkyl, acylaminoalkyl, alkoxycarbonyl, alkoxycarbonylamino, alkenyl, alkoxy, alkoxyalkyl, aryl, arylalkyl, aryloxy, aryloxyalkyl, cycloalkyl, cycloalkylalkyl, heterocyclyl, heterocyclylalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl, wherein any free hydroxyl group may be acylated to form $C(O)R_7$, and wherein both of R_{12} and R_{13} are not H; and

R_{14} represents substituted or unsubstituted aryl, arylalkyl, aryloxy, aryloxyalkyl, heteroaryl, heteroarylalkyl, heteroaryloxy, or heteroaryloxyalkyl.

27. The method of claim 26, wherein R_{11} represents substituted or unsubstituted arylalkyl.

28. The method of claim 27, wherein R_{11} represents substituted or unsubstituted benzyl.

29. The method of claim any one of claims 26-28, wherein L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2.

30. The method of claim 29, wherein L represents CH_2CH_2.

31. The method of any of claims 26-30, wherein each Y represents H.

32. The method of any of claims 26-31, wherein X represents S or CH=CH.

33. The method of claim 32, wherein X represents S.

34. The method of any of claims 26-33, wherein Z represents $R_3(CO)$.

35. The method of claim 34, wherein R_3 and R_{11} are not identical.

36. The method of any of claims 26-35, wherein R_1 and R_2 each represent H.

37. The method of claim 34, wherein R_3 represents substituted or unsubstituted arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl.

38. The method of claim 37, wherein R_3 represents substituted or unsubstituted heteroarylalkyl.

39. The method of claim 34, wherein R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl, heteroaryl or heteroaralkyl, R_9 represents H, and R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl.

40. The method of claim 39, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl, or heteroaryl.

41. The method of claim 39 or 40, wherein R_{10} represents hydroxy, hydroxyalkyl, or alkoxy.

42. The method of claim 26, wherein L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent

H, R_3 represents substituted or unsubstituted arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl, and R_{11} represents substituted or unsubstituted arylalkyl.

43. The method of claim 42, wherein R_3 represents substituted or unsubstituted heteroarylalkyl.

44. The method of claim 26, wherein L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl, heteroaryl or heteroaralkyl, R_9 represents H, R_{10} represents hydroxy, hydroxyalkyl, alkoxy or alkoxyalkyl, and R_{11} represents substituted or unsubstituted arylalkyl.

45. The method of claim 44, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl or heteroaryl.

46. The method of claim 45, wherein R_8 represents heteroaryl.

47. The method of any of claims 44-46, wherein R_{10} represents hydroxy, hydroxyalkyl or alkoxy.

48. The method of claim 26, wherein L represents CH_2CH_2, Y represents H, X represents S or CH=CH, Z represents $R_3(CO)$, R_1 and R_2 each represent H, R_3 represents substituted or unsubstituted arylalkyl, heteroarylalkyl, cycloalkyl or heterocycloalkyl, and R_{11} represents substituted or unsubstituted arylalkyl.

49. The method of claim 48, wherein R_3 represents substituted or unsubstituted heteroarylalkyl.

50. The method of claim 26, wherein L represents CH_2CH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents substituted or unsubstituted aryl, arylalkyl or heteroaryl, R_9 represents H, R_{10} represents hydroxy, hydroxyalkyl or alkoxy, and R_{11} represents substituted or unsubstituted arylalkyl.

51. The method of any preceding claim, wherein the level of pyruvate carboxylase is determined by measuring an amount of pyruvate carboxylase protein expressed in a sample of multiple myeloma cells from the patient.

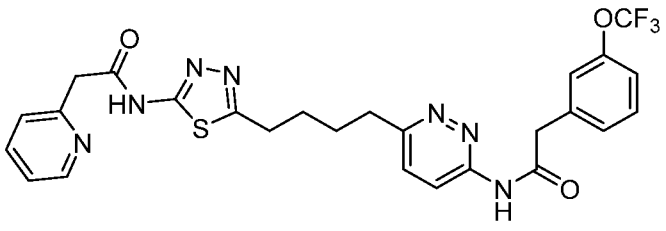
52. The method of claim 51, wherein the amount of pyruvate carboxylase protein is measured as western blot band intensity.

53. The method of claim 51, wherein the amount of pyruvate carboxylase protein is measured by immunohistochemistry.

54. The method of any one of claims 1-50, wherein the level of pyruvate carboxylase is determined by measuring the activity of pyruvate carboxylase.

55. The method of any of claims 1-50, wherein the level of pyruvate carboxylase is determined by whole transcriptome sequencing.

56. The method of any preceding claim, wherein the reference standard is a level of pyruvate carboxylase in a multiple myeloma cell line that is resistant to a compound of formula (II):



(II).

57. The method of claim 56, wherein the cell line is KMS-28PE or KMS-11 cells.

58. The method of claim 55, wherein the reference standard is expression of pyruvate carboxylase at a transcript count of about 220, wherein the transcript count is normalized against reference microarrays.

59. The method of any preceding claim, further comprising conjointly administering one or more additional chemotherapeutic agents.

60. The method of claim 59, wherein conjointly administering one or more additional chemotherapeutic agents provide improved efficacy relative to each individual administration of the glutaminase inhibitor or the one or more additional chemotherapeutic agent.

61. The method of claim 60, wherein conjointly administering one or more additional chemotherapeutic agents provide a synergistic effect.

62. The method of claim 60, wherein conjointly administering one or more additional chemotherapeutic agents provide an additive effect.

63. The method of any of claims 59-62, wherein the glutaminase inhibitor and the one or more additional chemotherapeutic agents are administered simultaneously.

64. The method of any of claims 59-62, wherein the one or more additional chemotherapeutic agents are administered within about 5 minutes to within about 168 hours prior to or after administration of the glutaminase inhibitor.

65. The method of any of claims 59-64, wherein the one or more additional chemotherapeutic agents are selected from ABT-263, aminoglutethimide, amsacrine, anastrozole, asparaginase, azacitidine, AZD5363, Bacillus Calmette–Guérin vaccine (bcg), bicalutamide, bisphosphonate, bleomycin, bortezomib, buserelin, busulfan, campothecin, capecitabine, carboplatin, carfilzomib, carmustine, chlorambucil, chloroquine, cisplatin, cladribine, clodronate, cobimetinib, colchicine, cyclophosphamide, cyproterone, cytarabine, dacarbazine, dactinomycin, daunorubicin, demethoxyviridin, dexamethasone, dichloroacetate, dienestrol, diethylstilbestrol, docetaxel, doxorubicin, epirubicin, eribulin, erlotinib, estradiol, estramustine, etoposide, everolimus, exemestane, filgrastim, fludarabine, fludrocortisone, fluorouracil (e.g., 5-fluorouracil), fluoxymesterone, flutamide, gemcitabine, genistein, goserelin, hydroxyurea, idarubicin, ifosfamide, imatinib, interferon, irinotecan, ixabepilone, lenalidomaide, letrozole, leucovorin, leuprolide, levamisole, lomustine, lonidamine, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, mesna, metformin, methotrexate, miltefosine, mitomycin, mitotane, mitoxantrone, MK-2206, nilutamide, nocodazole, octreotide,

oxaliplatin, olaparib, paclitaxel, pamidronate, pazopanib, pentostatin, perifosine, PF-04691502, plicamycin, pomalidomide, porfimer, procarbazine, raltitrexed, rituximab, romidepsin, rucaparib, selumetinib, sorafenib, streptozocin, sunitinib, suramin, talazoparib, tamoxifen, temozolomide, temsirolimus, teniposide, testosterone, thalidomide, thioguanine, thiotepa, titanocene dichloride, topotecan, trametinib, trastuzumab, tretinoin, veliparib, vinblastine, vincristine, vindesine, vinorelbine, and vorinostat (SAHA).

66. The method of claim 65, wherein the one or more additional chemotherapeutic agents are selected from bisphosphonate, bortezomib, carfilzomib, doxorubicin, lenalidomide, melphalan, pomalidomide, and vincristine.

67. The method of any of claims 59-64, wherein the one or more additionanl chemotherapeutic agents are immunooncology therapeutic agents selected from abagovomab, adecatumumab, afutuzumab, anatumomab mafenatox, apolizumab, blinatumomab, catumaxomab, durvalumab, epratuzumab, indoximod, inotuzumab ozogamicin, intelumumab, ipilimumab, isatuximab, lambrolizumab, nivolumab, ocaratuzumab, olatatumab, pembrolizumab, pidilizumab, ticilimumab, samalizumab, and tremelimumab

68. The method of any preceding claim, further comprising conjointly administering one or more immunomodulatory agents.

69. The method of claim 68, wherein conjointly administering one or more immunomodulatory agents provide improved efficacy relative to each individual administration of the glutaminase inhibitor or the one or more immunomodulatory agent.

70. The method of claim 69, wherein conjointly administering one or more immunomodulatory agents provide a synergistic effect.

71. The method of claim 69, wherein conjointly administering one or more immunomodulatory agents provide an additive effect.

72. The method of any of claims 68-71, wherein the glutaminase inhibitor and the one or more immunomodulatory agents are administered simultaneously.

73. The method of any of claims 68-71, wherein the one or more immunomodulatory agents are administered within about 5 minutes to within about 168 hours prior to or after administration of the glutaminase inhibitor.

74. The method of any of claims 68-73, wherein the one or more immunomodulatory agents are selected from granulocyte colony-stimulating factor (G-CSF), interferons, imiquimod, IL-2, IL-7, IL-12, various chemokines, synthetic cytosine phosphate-guanosine (CpG) oligodeoxynucleotides, glucans, and synthetic small molecules such as apremilast (CC-10004), CC-122, CC-11006, CC-10015, dexamethasone, prednisone, ImiDs, lenalidomide, pomalidomide, and thalidomide.

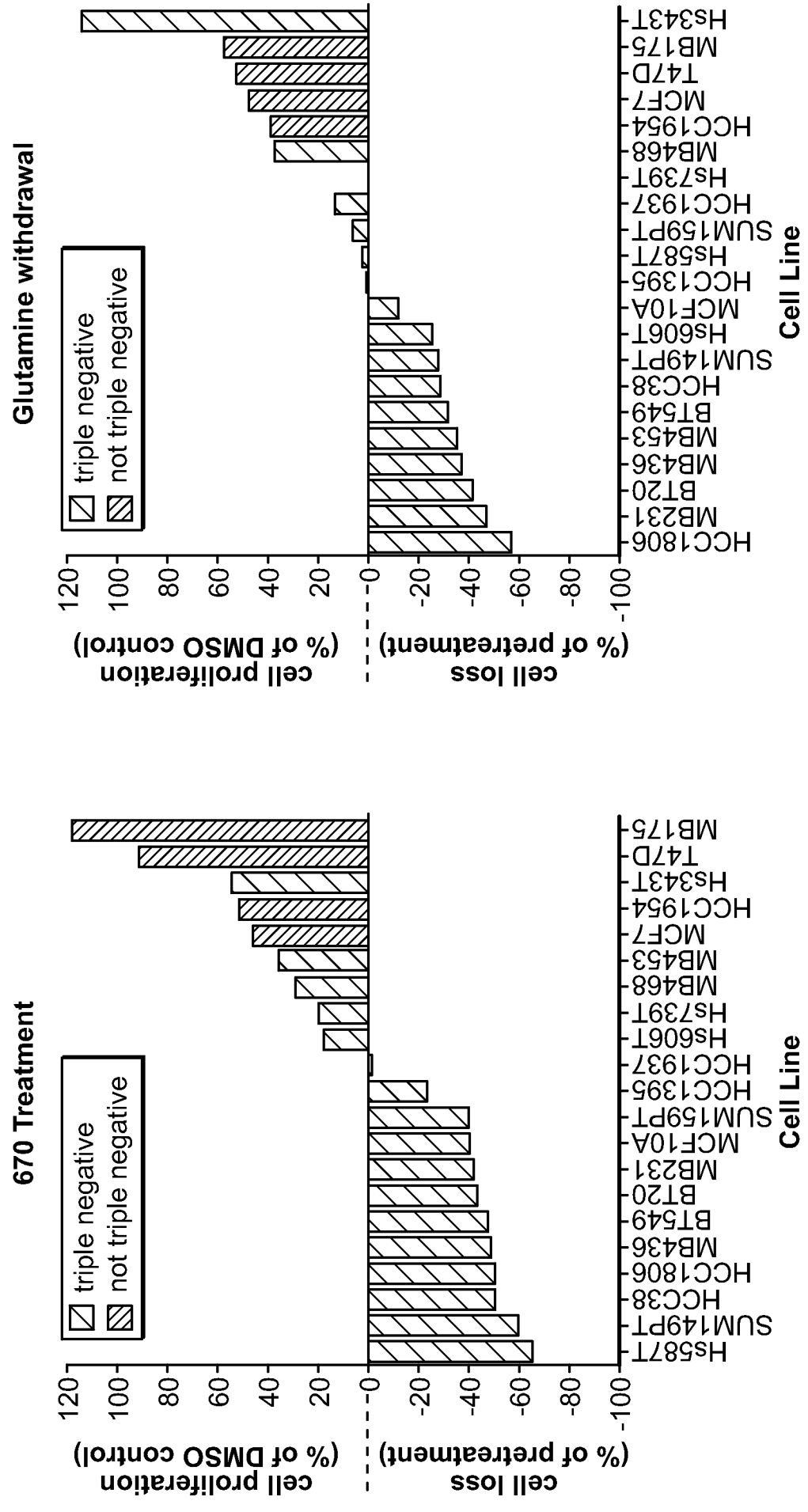
75. The method of claim 74, wherein the immunomodulatory agent is selected from dexamethasone, prednisone, and IMiDs.

76. The method of any preceding claim, further comprising conjointly administering one or more anticancer agents selected from enzyme inhibitors (such as a kinase inhibitor), mitotic inhibitors, DNA-modifying agents, and cytidine analogs.

77. The method of claim 74, wherein the anti-cancer agent is selected from microtubule assembly inhibitors, AKT inhibitors, mTOR inhibitors, MEK inhibitors, RTK inhibitors, ATM inhibitors, ATR inhibitors, PI3K inhibitors, EGFR inhibitors, B-Raf inhibitors, C-kit inhibitors, PARP inhibitors, DNA cross-linking agents, DNA intercalating agents, and cytidine analogs.

78. The method of claim 75, wherein the anti-cancer agent is selected from vincristine, carboplatin, cisplatin, gemcitabine, MK2206, everolimus, trametinib, sunitinib, sorafenib, BEZ235, paclitaxel, docetaxel, erlotinib, selumetinib, sirolimus, trametinib, temsirolimus, pazopanib, olaparib, and GSK1120212.

Figure 1


670 Treatment
triple negative
not triple negative
cell proliferation (% of DMSO control)
cell loss (% of pretreatment)
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
Cell Line
HS578T
SUM149PT
HCC38
HCC1806
MB436
BT549
BT20
MB231
MCF10A
SUM159PT
HCC1395
HCC1937
HS606T
HS739T
MB468
MB453
MCF7
HCC1954
HS343T
T47D
MB175
Glutamine withdrawal
triple negative
not triple negative
cell proliferation (% of DMSO control)
cell loss (% of pretreatment)
Cell Line
HCC1806
MB231
BT20
MB436
MB453
BT549
HCC38
SUM149PT
HS606T
MCF10A
HCC1395
HS578T
SUM159PT
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HS739T
MB468
HCC1954
MCF7
T47D
MB175
HS343T

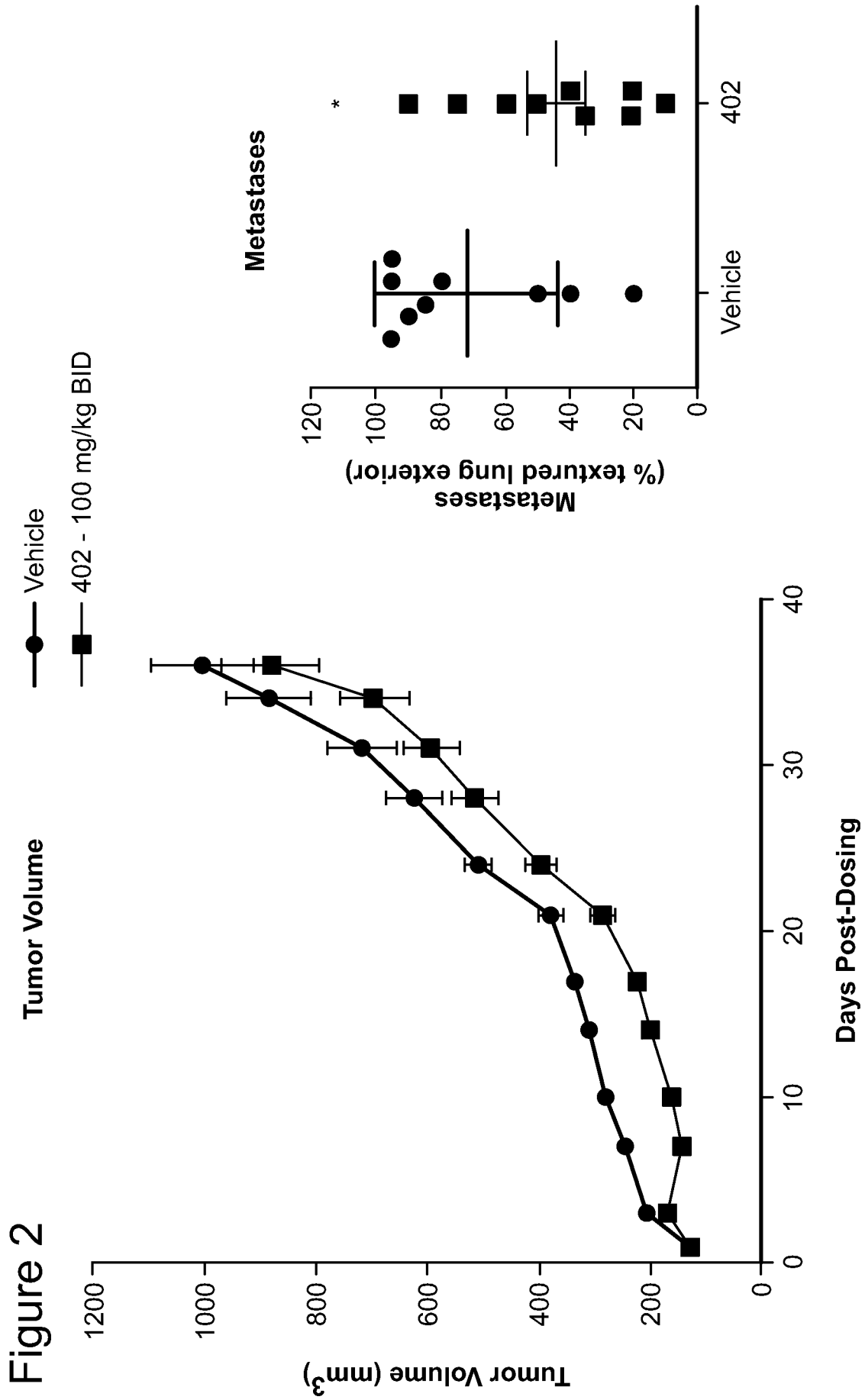

Tumor Volume
Vehicle
402 - 100 mg/kg BID
Tumor Volume (mm3)
0
200
400
600
800
1000
1200
0
10
20
30
40
Days Post-Dosing
Metastases
Metastases (% textured lung exterior)
0
20
40
60
80
100
120
*
Vehicle
402

Figure 2

Figure 3

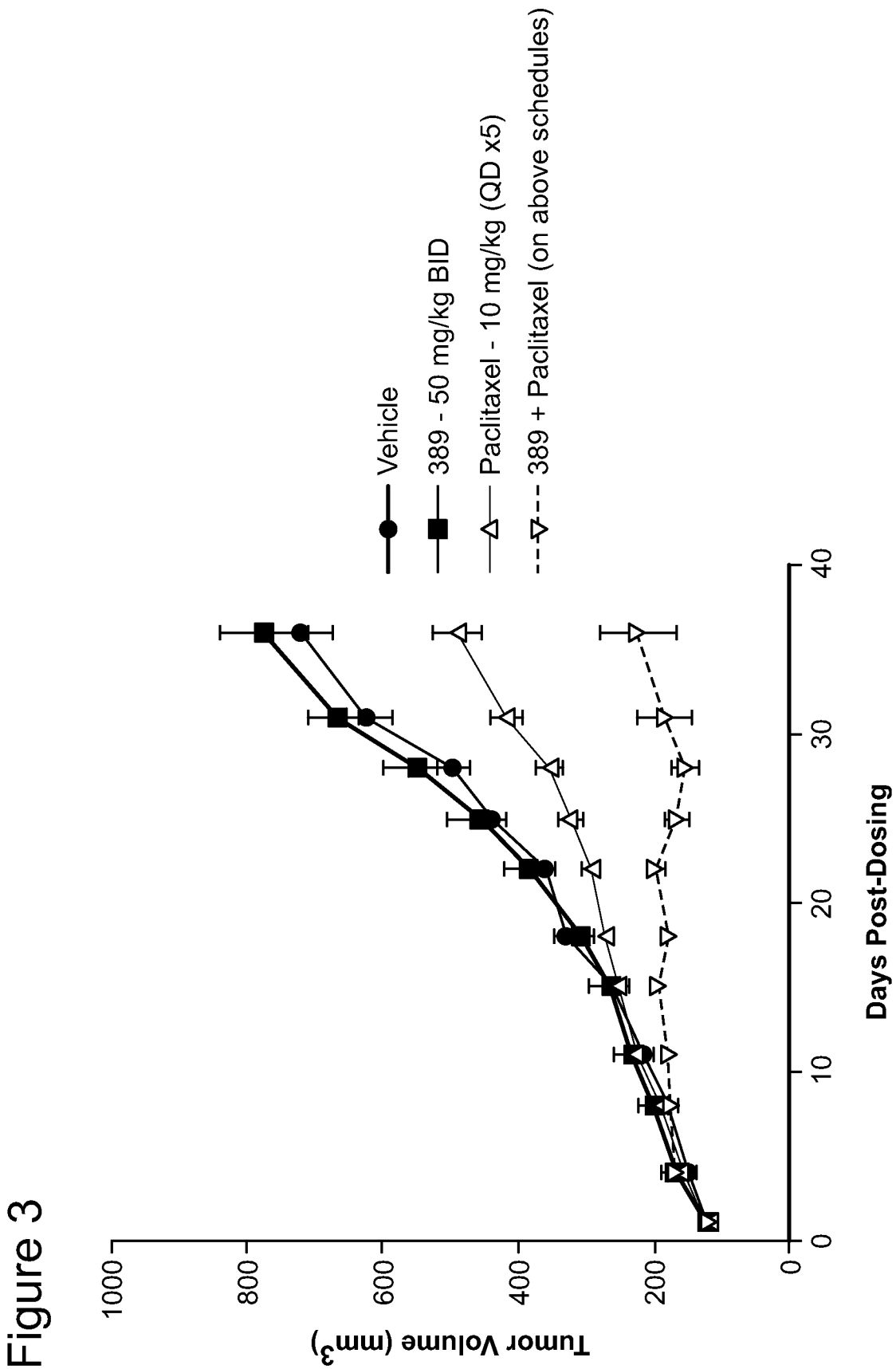

Vehicle
389 - 50 mg/kg BID
Paclitaxel - 10 mg/kg (QD x5)
389 + Paclitaxel (on above schedules)
Tumor Volume (mm3)
Days Post-Dosing
0
200
400
600
800
1000
0
10
20
30
40

Figure 4

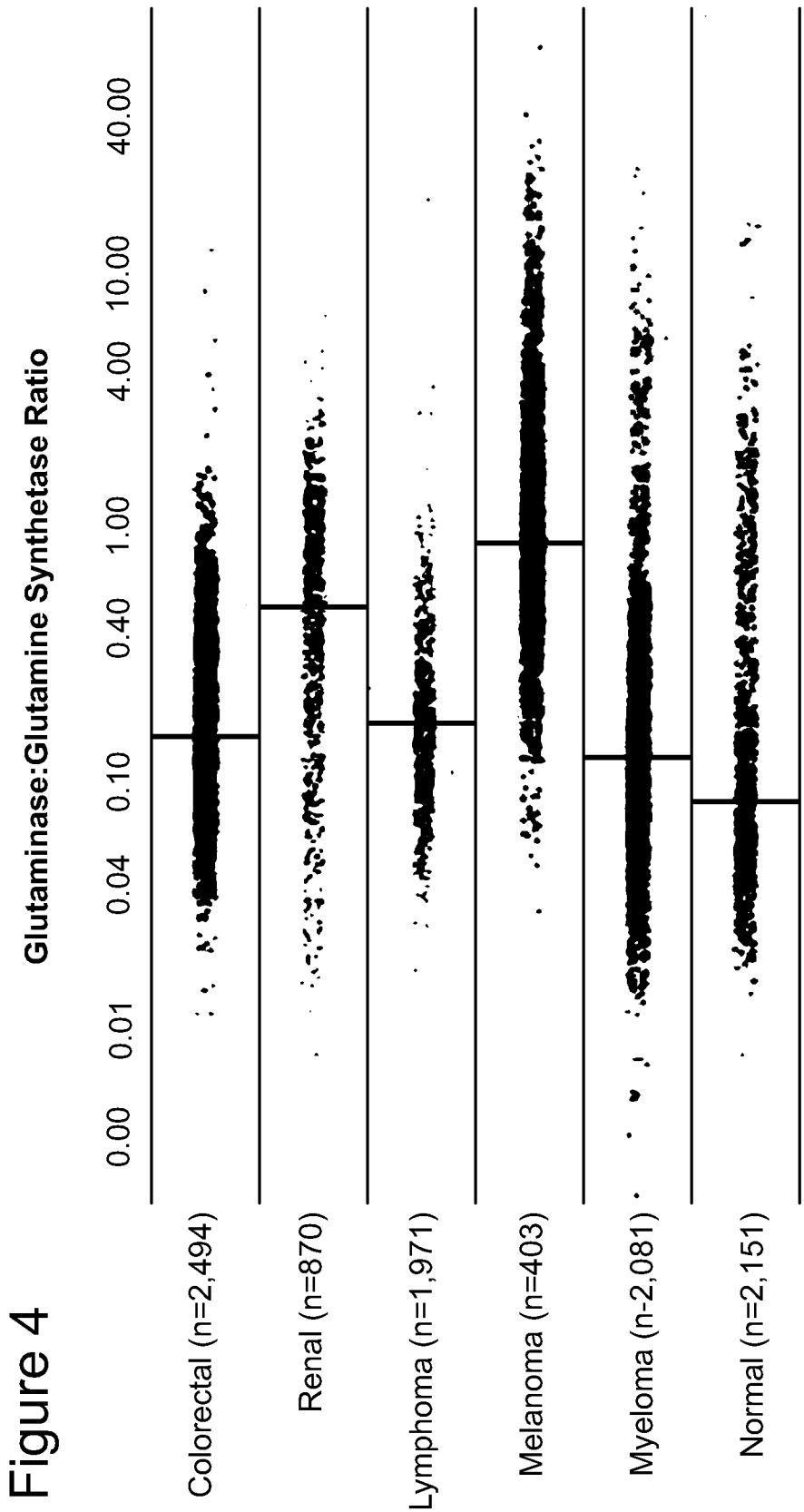

Glutaminase:Glutamine Synthetase Ratio
0.00
0.01
0.04
0.10
0.40
1.00
4.00
10.00
40.00
Colorectal (n=2,494)
Renal (n=870)
Lymphoma (n=1,971)
Melanoma (n=403)
Myeloma (n-2,081)
Normal (n=2,151)

Figure 5

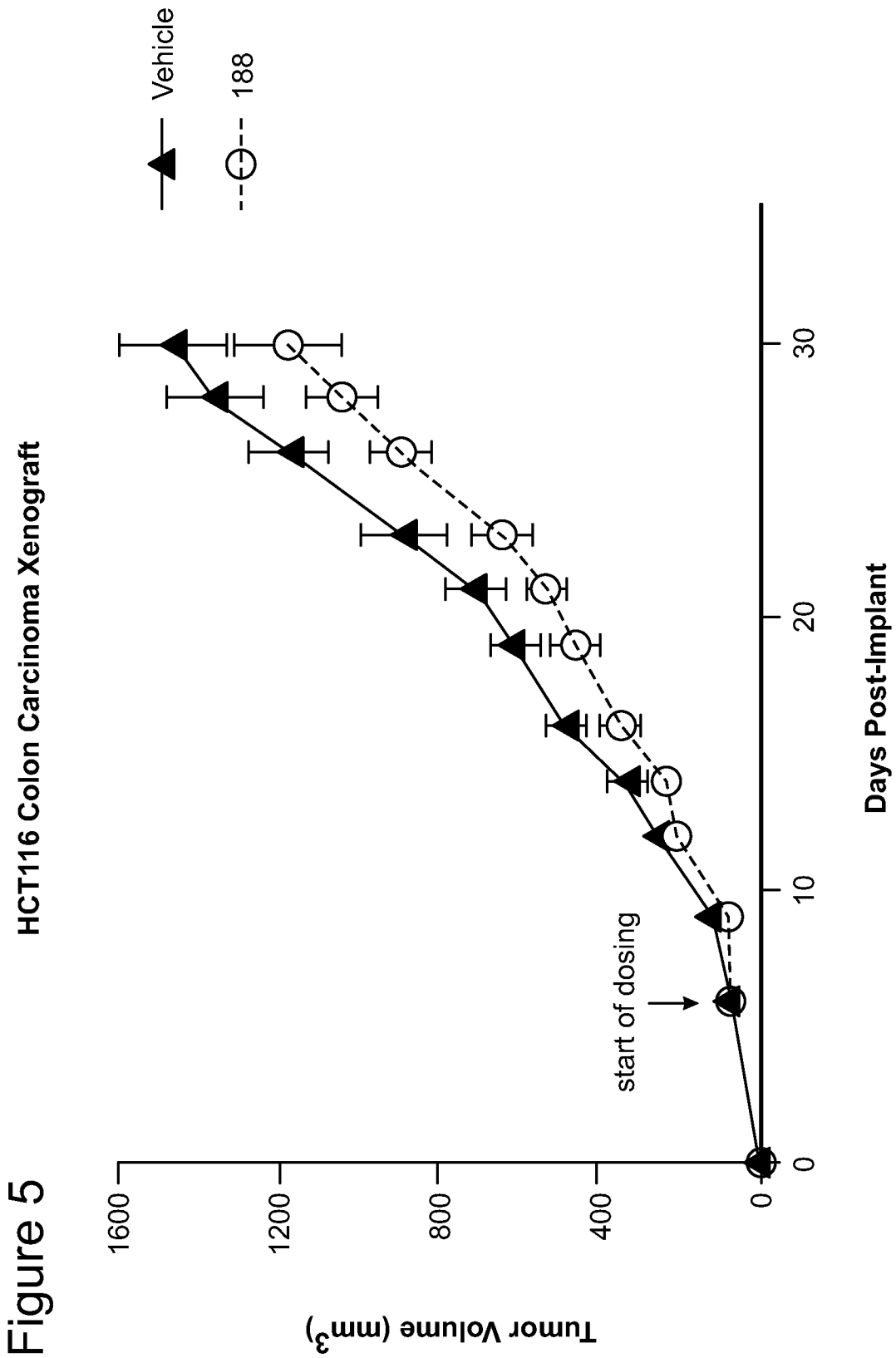

HCT116 Colon Carcinoma Xenograft
Vehicle
188
start of dosing
Tumor Volume (mm3)
Days Post-Implant
0
400
800
1200
1600
0
10
20
30

Figure 6

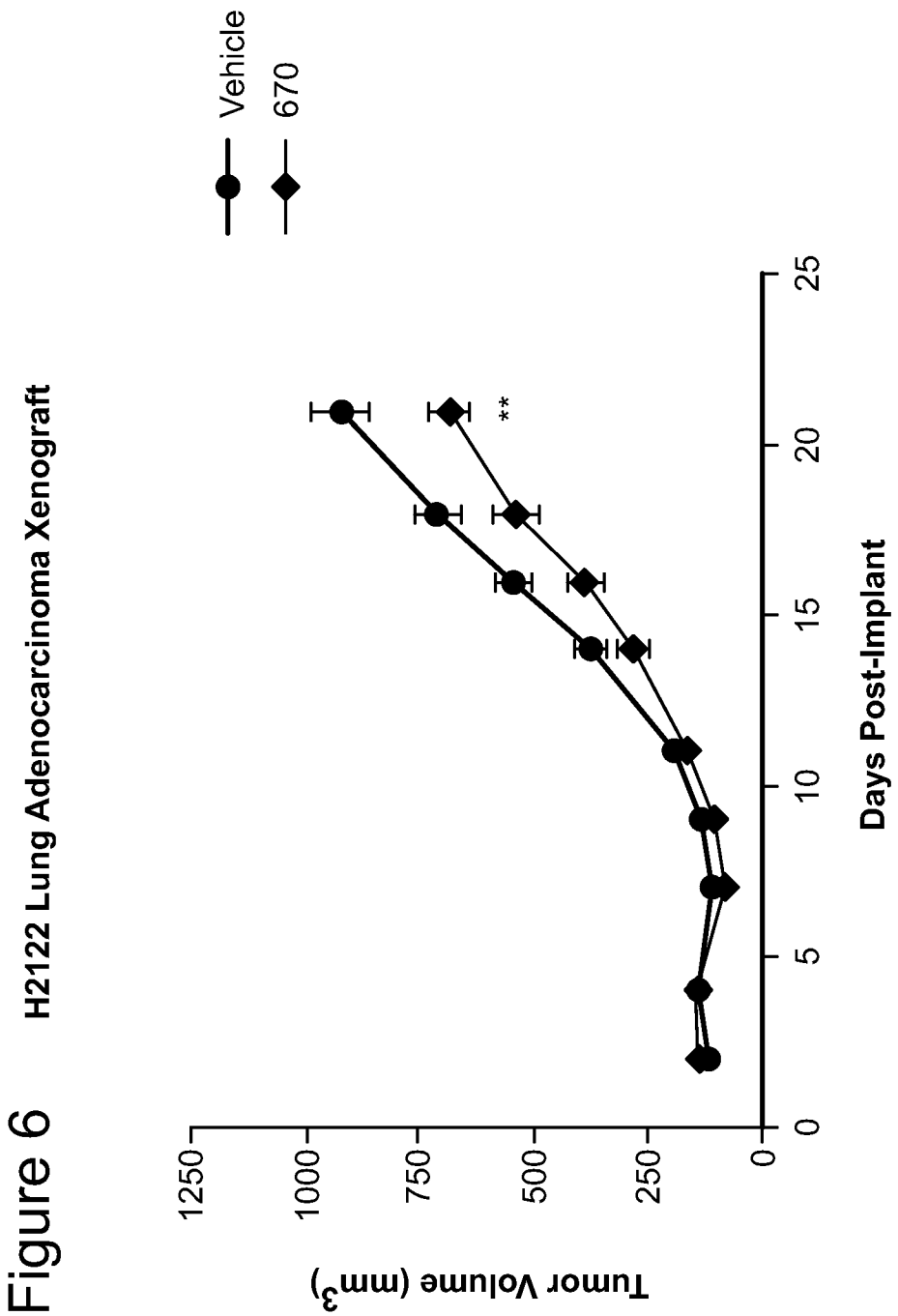

H2122 Lung Adenocarcinoma Xenograft
Vehicle
670
**
Tumor Volume (mm3)
1250
1000
750
500
250
0
0
5
10
15
20
25
Days Post-Implant

Figure 7

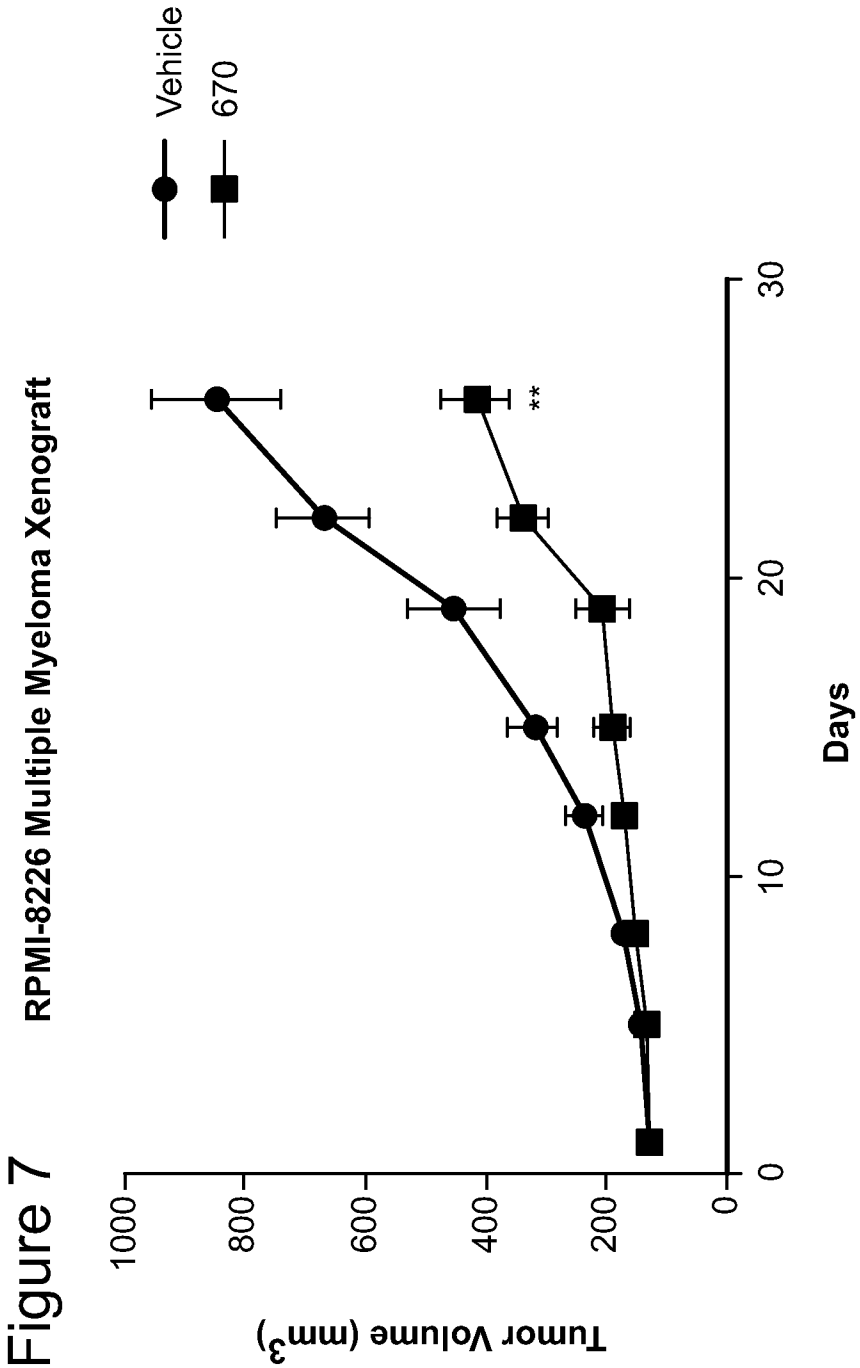
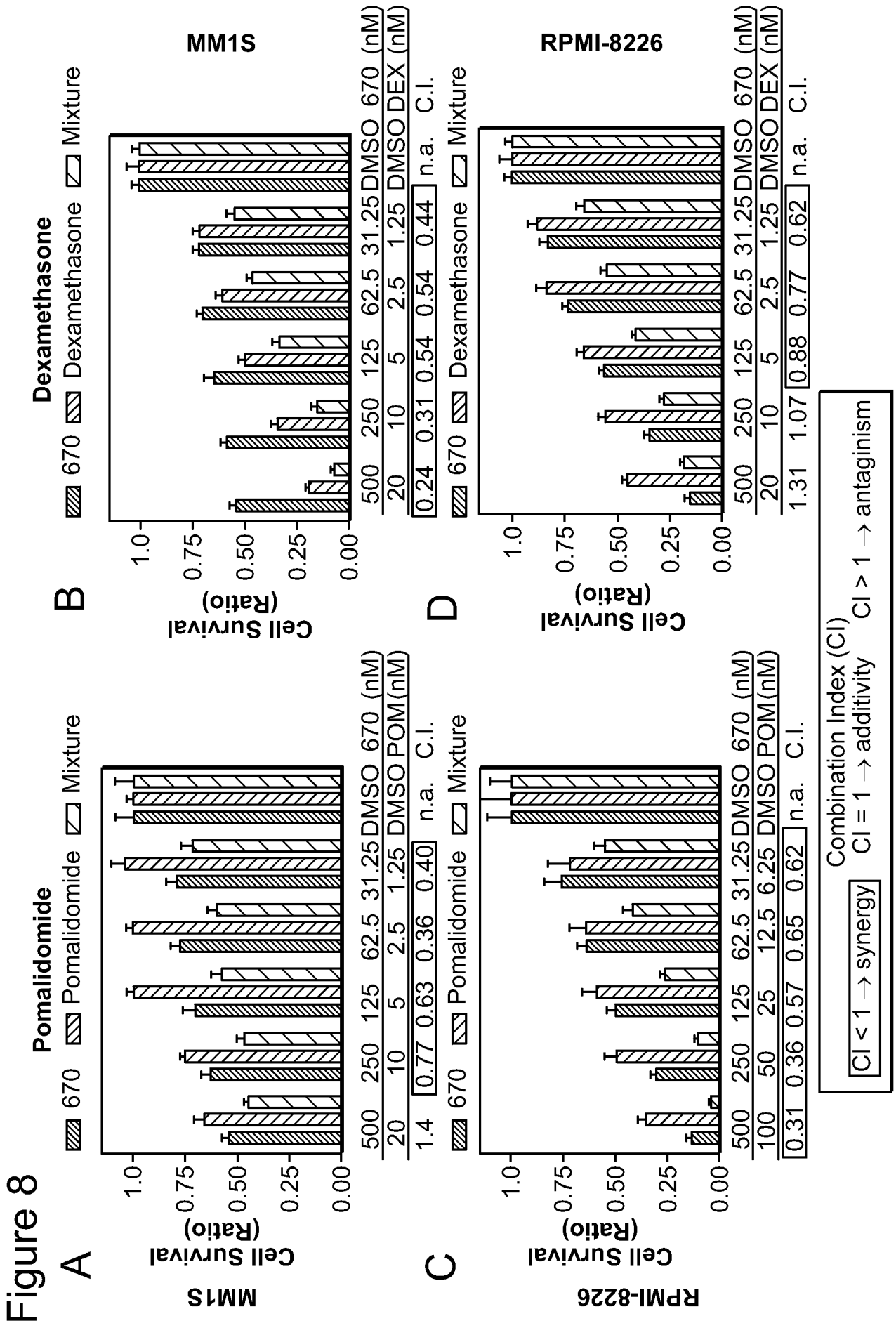

RPMI-8226 Multiple Myeloma Xenograft
Vehicle
670
**
Tumor Volume (mm3)
1000
800
600
400
200
0
0
10
20
30
Days

Figure 8


A
MM1S
Cell Survival (Ratio)
1.0
0.75
0.50
0.25
0.00
Pomalidomide
670
Pomalidomide
Mixture
500 250 125 62.5 31.25 DMSO 670 (nM)
20 10 5 2.5 1.25 DMSO POM(nM)
1.4 0.77 0.63 0.36 0.40 n.a. C.I.
B
MM1S
Cell Survival (Ratio)
Dexamethasone
670
Dexamethasone
Mixture
500 250 125 62.5 31.25 DMSO 670 (nM)
20 10 5 2.5 1.25 DMSO DEX (nM)
0.24 0.31 0.54 0.54 0.44 n.a. C.I.
C
RPMI-8226
Cell Survival (Ratio)
670
Pomalidomide
Mixture
500 250 125 62.5 31.25 DMSO 670 (nM)
100 50 25 12.5 6.25 DMSO POM(nM)
0.31 0.36 0.57 0.65 0.62 n.a. C.I.
D
RPMI-8226
Cell Survival (Ratio)
670
Dexamethasone
Mixture
500 250 125 62.5 31.25 DMSO 670 (nM)
20 10 5 2.5 1.25 DMSO DEX (nM)
1.31 1.07 0.88 0.77 0.62 n.a. C.I.
Combination Index (CI)
CI < 1 → synergy
CI = 1 → additivity
CI > 1 → antaginism

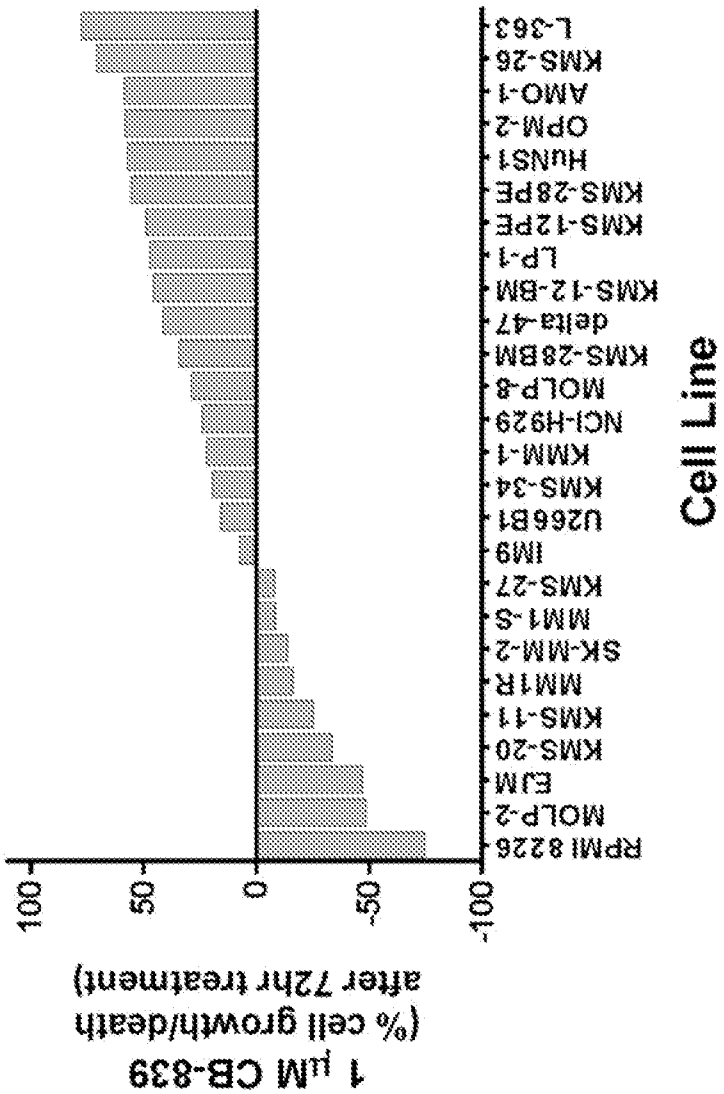

1 μM CB-839
(% cell growth/death
after 72hr treatment)
100
50
0
-50
-100
Cell Line
RPMI 8226
MOLP-2
EJM
KMS-20
KMS-11
MM1R
SK-MM-2
MM1-S
KMS-27
IM9
U266B1
KMS-34
KMM-1
NCI-H929
MOLP-8
KMS-28BM
delta-47
KMS-12-BM
LP-1
KMS-12PE
KMS-28PE
HuNS1
OPM-2
AMO-1
KMS-26
L-363

Figure 9

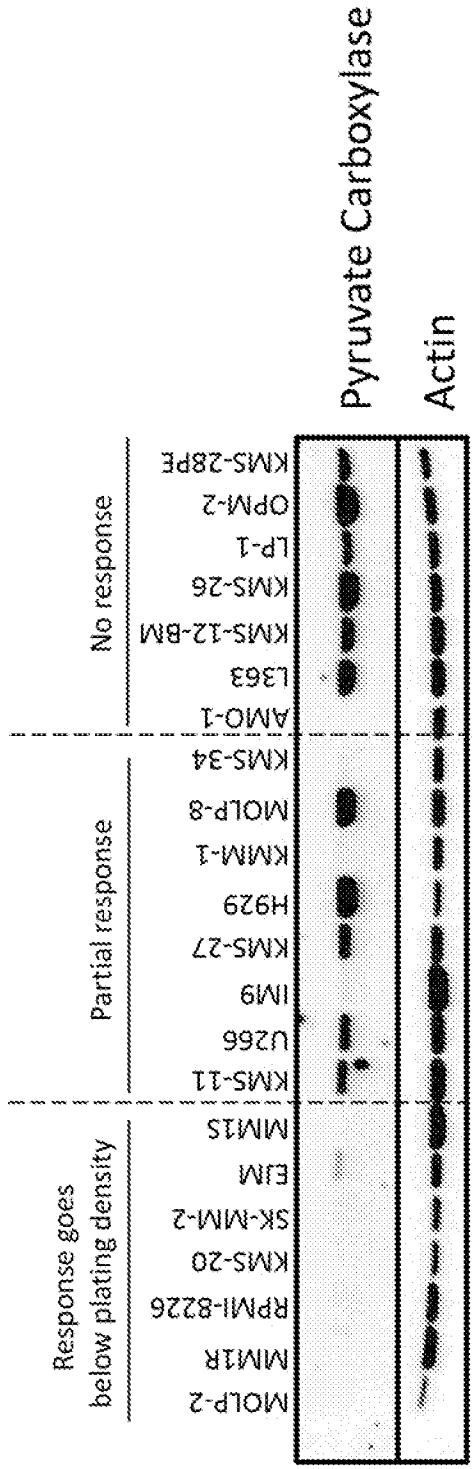

Pyruvate Carboxylase
Actin
Response goes below plating density
MOLP-2
MM1R
RPMI-8226
KMS-20
SK-MM-2
EJM
MM1S
Partial response
KMS-11
U266
IM9
KMS-27
H929
KMM-1
MOLP-8
KMS-34
No response
AMO-1
L363
KMS-12-BM
KMS-26
LP-1
OPM-2
KMS-28PE

Figure 10

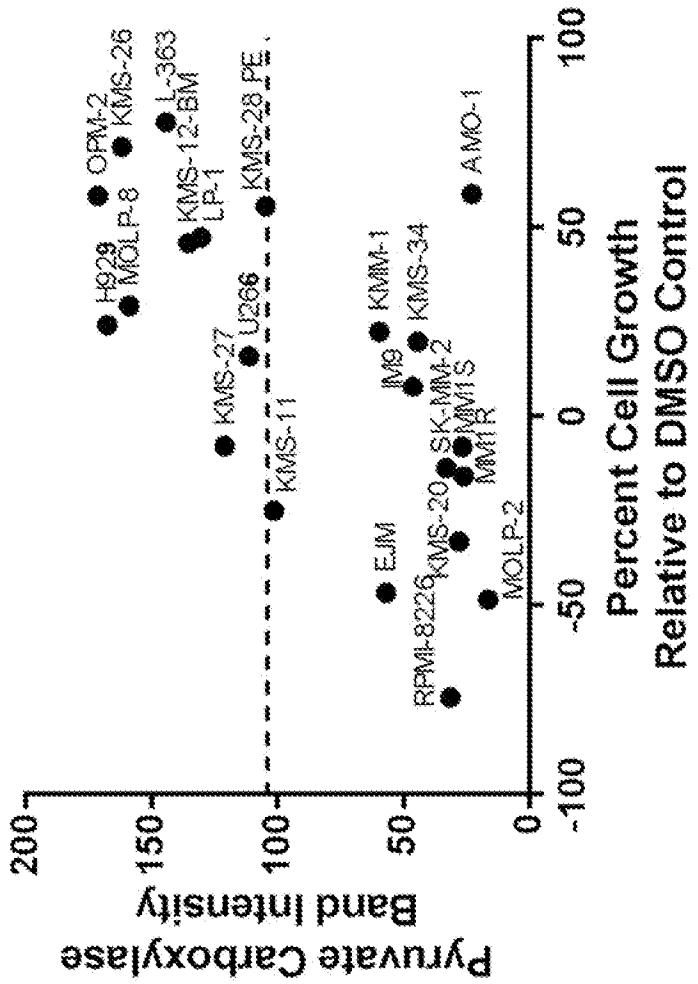

Pyruvate Carboxylase
Band Intensity
200
150
100
50
0
-100
-50
0
50
100
Percent Cell Growth
Relative to DMSO Control
H929
MOLP-8
OPM-2
KMS-26
L-363
KMS-12-BM
LP-1
KMS-28 PE
KMS-27
U266
KMS-11
EJM
RPMI-8226
KMS-20
MOLP-2
IM9
KMM-1
KMS-34
SK-MM-2
MM1S
MM1R
AMO-1

Figure 11

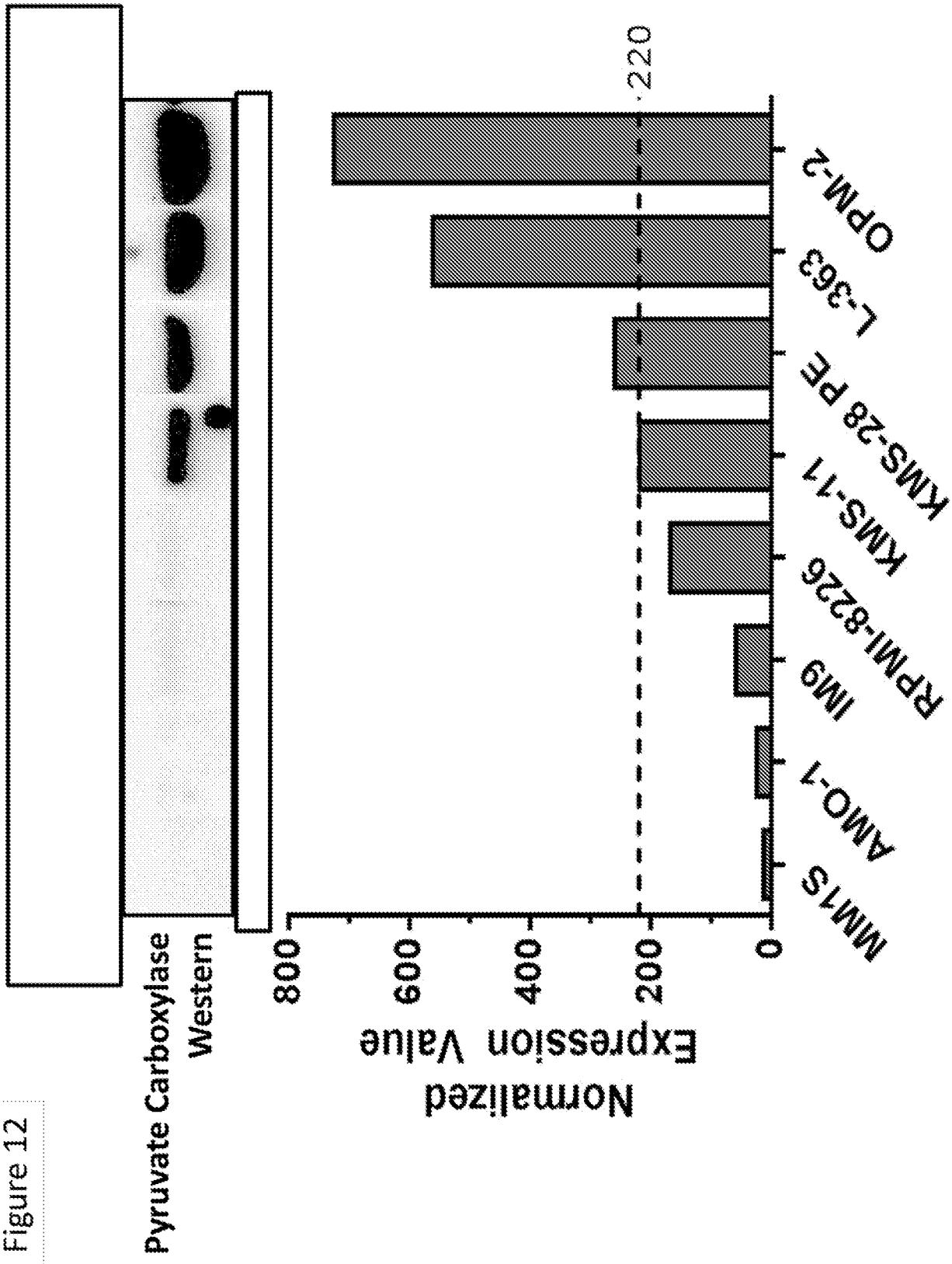

Pyruvate Carboxylase Western
Normalized Expression Value
800
600
400
200
0
220
MM1S
AMO-1
IM9
RPMI-8226
KMS-11
KMS-28 PE
L-363
OPM-2

Figure 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/041891

A. CLASSIFICATION OF SUBJECT MATTER

IPC (2015.01) C07D 417/08, C07D 417/14, C07D 413/14, A61K 31/424500, A61K 31/433, A61P 35/00, G01N 33/48

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 (2015.01) C07D 417/08, C07D 417/14, C07D 413/14, A61K 31/424500, A61K 31/433, A61P 35/00, G01N 33/48

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases consulted: THOMSON INNOVATION, CAPLUS, BIOSIS, EMBASE, MEDLINE, Google Scholar
Search terms used: pyruvate carboxylase, glutaminase inhibitor, cancer, multiple myeloma, protein, western blot band, immunohistochemistry, sequencing, synergistic, immunomodulator

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014079150 A1 AGIOS PHARMACEUTICALS INC [US] LEMIEUX RENE M [US] 30 May 2014 (2014/05/30) abstract, pages 2-4, 75-79, 87, 90-93	1-3,6,8-11,51-55,59, 65,66,68,74,76-78
Y	abstract, pages 2-4, 75-79, 87, 90-93	1-55,59-78
Y	WO 2014089048 A1 CALITHERA BIOSCIENCES INC [US]; BENNETT MARK K [US]; GROSS MATTHEW I [US]; BROMLEY SUSAN D [US]; LI JIM [US]; CHEN LIJING [US]; GOYAL BINDU [US]; LAIDIG GUY [US]; STANTON TIMOTHY FRIEND [US]; SJOGREN ERIC BRIAN [US] 12 Jun 2014 (2014/06/12) pages 3, 5-7, 22-23, 32, 58, Fig. 18, examples 17, 18, claims 1-49, 61, 67-75	1-55,59-78
A	US 2014142081 A1 LEMIEUX RENE M [US]; POPOVICI-MULLER JANETA [US]; SALITURO FRANCESCO G [US]; SAUNDERS JEFFREY O [US]; TRAVINS JEREMY [US]; CHEN YONGSHENG [CN]; AGIOS PHARMACEUTICALS INC [US] 22 May 2014 (2014/05/22) whole document	1-3,6,8-11,51-55,59, 65,66,68,74,76-78

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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"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	Date of mailing of the international search report
04 Nov 2015	04 Nov 2015
Name and mailing address of the ISA: Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Facsimile No. 972-2-5651616	Authorized officer YUDBOROVSKI Evgenia Telephone No. 972-2-5651654

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/041891

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2014142146 A1 LEMIEUX RENE M [US]; POPOVICI-MULLER JANETA [US]; SALITURO FRANCESCO G [US]; SAUNDERS JEFFREY O [US]; TRAVINS JEREMY [US]; YAN SHUNQI [US]; AGIOS PHARMACEUTICALS INC [US] 22 May 2014 (2014/05/22) whole document	1-3,6,8-11,51-55,59, 65,66,68,74,76-78
A	NAMBA, Masayoshi, et al. Establishment of five human myeloma cell lines. In vitro cellular & developmental biology, 1989, 25.8: 723-729. NAMBA, Masayoshi, et al. 31 Aug 1989 (1989/08/31) page 723 "introduction"	56,57

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2015/041891

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
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		CN 104936954 A	23 Sep 2015
		CR 20150316 A	28 Aug 2015
		EP 2922850 A1	30 Sep 2015
		KR 20150085079 A	22 Jul 2015
		PE 10722015 A1	26 Aug 2015
		TW 201427963 A	16 Jul 2014
		US 2014142081 A1	22 May 2014
		US 9029531 B2	12 May 2015
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		WO 2014079011 A1	30 May 2014
		WO 2014079136 A1	30 May 2014
WO 2014089048 A1	12 Jun 2014	WO 2014089048 A1	12 Jun 2014
		AU 2013356241 A1	09 Jul 2015
		CA 2892817 A1	12 Jun 2014
		EP 2925318 A1	07 Oct 2015
		KR 20150091389 A	10 Aug 2015
		US 2015004134 A1	01 Jan 2015
US 2014142081 A1	22 May 2014	US 2014142081 A1	22 May 2014
		US 9029531 B2	12 May 2015
		AU 2013347771 A1	11 Jun 2015
		CA 2893510 A1	30 May 2014
		CN 104936954 A	23 Sep 2015
		CR 20150316 A	28 Aug 2015
		EP 2922850 A1	30 Sep 2015
		KR 20150085079 A	22 Jul 2015
		PE 10722015 A1	26 Aug 2015

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US2015/041891

Patent document cited search report	Publication date	Patent family member(s)	Publication Date
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		US 2015291576 A1	15 Oct 2015
		WO 2014079011 A1	30 May 2014
		WO 2014079136 A1	30 May 2014
		WO 2014079150 A1	30 May 2014
US 2014142146 A1	22 May 2014	US 2014142146 A1	22 May 2014
		AU 2013347933 A1	11 Jun 2015
		AU 2013347933 A2	09 Jul 2015
		CA 2892089 A1	30 May 2014
		EP 2922832 A1	30 Sep 2015
		KR 20150085078 A	22 Jul 2015
		US 2015299152 A1	22 Oct 2015
		WO 2014081925 A1	30 May 2014