Combination Therapy with Glutaminase Inhibitors

Related Applications

This application claims the benefit of priority to U.S. Provisional Patent Application serial number 62/012,061, filed June 13, 2014, and U.S. Provisional Patent Application serial number 62/149,487, filed April 17, 2015, the contents of which are hereby incorporated by reference.

Background

It has been observed that cancer cells rely on exogenous glutamine, albeit the degree of dependency varies from cancer to cancer. In these actively proliferating cancer 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, functioning as a control point for glutamine metabolism, GLS may provide a potential new target for the treatment of cancer. Recently, the creation of GLS inhibitors that are specific and capable of being formulated for in vivo use is permitting this hypothesis to be tested. Therapeutic approaches for clinical use of these compounds would be advantageous.

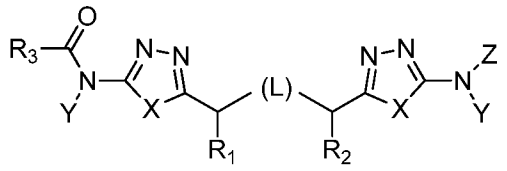
Summary of Invention

The present invention provides a method of treating or preventing cancer, myelodysplastic syndrome (MDS), a myelproliferative disease, an immunological disease, or a neurological disease, comprising conjointly administering a glutaminase inhibitor and an anticancer agent selected from an enzyme inhibitor (such as a kinase inhibitor), a mitotic inhibitor, a DNA-modifying agent, and a cytidine analog.

In certain embodiments, the anticancer 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.

In certain embodiments, the anticancer 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.

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, the cancer is selected from biliary cancer, breast cancer, colorectal cancer, leukemia, acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), hairy cell leukemia, T-cell leukemia, brain malignancy, lymphoma, diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, Hodgkin's lymphoma, MALT lymphoma, mantle cell lymphoma (MCL), no-Hodgkin lymphoma (NHL), endometrial cancer, head and neck cancers, Kaposi's sarcoma, lung cancer, melanoma, multiple myeloma (MM), myelodisplastic disease (MDS), ocular disease, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, thyroid cancer, tuberous sclerosis, and Waldenstrom macrogloulinemia (WM).

In certain embodiments, the myeloproliferative disease is selected from chronic eosinophilic leukemia, chronic myelogenous leukemia (CML), chronic neutrophilic leukemia, essential thrombocythemia, polycythemia vera, and myelofibrosis.

In certain embodiments, the immune-related disease is selected from ankylosing spondylitis, Crohn's disease, erythema nodosum leprosum (ENL), graft versus host disease (GVHD), HIV-associated wasting syndrome, lupus

erythematosus, organ transplant rejection, post-polycythemia, psoriasis, psoriatic arthritis, recurrent aphthous ulcers, rheumatoid arthritis (RA), severe recurrent aphthous stomatitis, systemic sclerosis, and tuberous sclerosis.

In certain embodiments, the present invention provides a pharmaceutical preparation suitable for use in a human patient in the treatment or prevention of cancer, myelodisplastic syndrome (MDS), myelproliferative disease or immune-related diseases, comprising an effective amount of an invention glutaminase inhibitor (such as a compound of formula I), an anticancer agent selected from an enzyme inhibitor (such as a kinase inhibitor), a mitotic inhibitor, a DNA-modifying agent, and a cytidine analog, 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 other embodiments, the present invention provides a kit for the treatment or prevention of cancer, myelodisplastic syndrome (MDS), myelproliferative disease, immunological diseases, or neurological diseases, comprising an effective amount of an anticancer agent selected from an enzyme inhibitor (such as a kinase inhibitor), a mitotic inhibitor, a DNA-modifying agent, and a cytidine analog and an effective amount of a glutaminase inhibitor (such as a compound of formula I), wherein the anticancer agent and inhibitor are optionally formulated as pharmaceutical compositions, either separately or in combination. In certain embodiments, the kits may be for use in treating or preventing a condition or disease as described herein.

Detailed Description of the Drawings

FIG. 1, which consists of panels A-C, contains a series of bar graphs demonstrating synergy between compound CB-839 and vincristine at various concentrations of compound CB-839 in multiple myeloma cell lines. Compound CB-839 has the chemical structure of Formula (II).

FIG. 2, which consists of panels A-C, contains a series of bar graphs demonstrating strong synergy between compound CB-839 and AKT inhibitor MK2206 at various concentrations of compound CB-839 in multiple myeloma cell lines.

FIG. 3, which consists of panels A-C, contains a series of bar graphs demonstrating strong synergy between compound CB-839 and mTOR inhibitor everolimus at various concentrations of compound CB-839 in multiple myeloma cell lines.

FIG. 4, which consists of panels A-C, contains a series of bar graphs demonstrating strong synergy between compound CB-839 and MEK inhibitor trametinib at various concentrations of compound CB-839 in multiple myeloma cell lines.

FIG. 5, which consists of panels A-C, contains a series of bar graphs demonstrating strong synergy between compound CB-839 and RTK inhibitor sorafenib at various concentrations of compound CB-839 in multiple myeloma cell lines.

FIG. 6, which consists of panels A-C, contains a series of bar graphs demonstrating therapeutic interaction between compound CB-839 and carboplatin at various concentrations of compound CB-839 in triple negative breast cancer cell lines.

FIG. 7, which consists of panels A-C, contains a series of bar graphs demonstrating an additive effect between compound CB-839 and cytidine analog gemcitabine at various concentrations of compound CB-839 in triple negative breast cancer cell lines.

FIG. 8, which consists of panels A-C, contains a series of bar graphs demonstrating therapeutic interaction between compound CB-839 and doxorubicin at various concentrations of compound CB-839 in triple negative breast cancer cell lines.

FIG. 9, which consists of panels A and B, contains a series of bar graphs demonstrating synergy between compound CB-839 and mTOR inhibitor everolimus at various concentrations of compound CB-839 in triple negative breast cancer cell lines.

FIG. 10, which consists of panels A and B, contains a series of bar graphs demonstrating synergy between compound CB-839 and AKT inhibitor MK2206 at various concentrations of compound CB-839 in triple negative breast cancer cell lines.

FIG. 11, which consists of panels A-F, contains a series of bar graphs demonstrating synergy between compound CB-839 and EGFR inhibitor erlotinib at various concentrations of compound CB-839 in lung cancer cell lines.

FIG. 12, which consists of panels A-E, contains a series of bar graphs demonstrating synergy between compound CB-839 and MEK inhibitor trametinib at various concentrations of compound CB-839 in lung cancer cell lines.

FIG. 13, which consists of panels A-E, contains a series of bar graphs demonstrating synergy between compound CB-839 and mTOR inhibitor everolimus at various concentrations of compound CB-839 in lung cancer cell lines.

FIG. 14, which consists of panels A-C, contains a series of bar graphs demonstrating synergy between compound CB-839 and mTOR inhibitor temsirolimus at various concentrations of compound CB-839 in lung cancer cell lines.

FIG. 15, which consists of panels A-E, contains a series of bar graphs demonstrating synergy between compound CB-839 and AKT inhibitor MK2206 at various concentrations of compound CB-839 in lung cancer cell lines.

FIG. 16, which consists of panels A and B, contains a series of bar graphs demonstrating synergy between compound CB-839 and PI3K/mTOR inhibitor BEZ235 at various concentrations of compound CB-839 in lung cancer cell lines.

FIG. 17, which consists of panels A-E, contains a series of bar graphs demonstrating synergy between compound CB-839 and RTK inhibitor sorafenib at various concentrations of compound CB-839 in renal cell carcinoma cell lines.

FIG. 18, which consists of panels A-E, contains a series of bar graphs demonstrating synergy between compound CB-839 and C-kit and pan-RTK inhibitor sunitinib at various concentrations of compound CB-839 in renal cell carcinoma cell lines.

FIG. 19, which consists of panels A-C, contains a series of bar graphs demonstrating synergy between compound CB-839 and pan-RTK inhibitor pazopanib at various concentrations of compound CB-839 in renal cell carcinoma cell lines.

FIG. 20, which consists of panels A-C, contains a series of bar graphs demonstrating synergy between compound CB-839 and mTOR inhibitor everolimus at various concentrations of compound CB-839 in renal cell carcinoma cell lines.

FIG. 21, which consists of panels A-C, contains a series of bar graphs demonstrating synergy between compound CB-839 and mTOR inhibitor temsirolimus at various concentrations of compound CB-839 in renal cell carcinoma cell lines.

FIG. 22, which consists of panels A-C, contains a series of bar graphs demonstrating synergy between compound CB-839 and MEK inhibitor trametinib at various concentrations of compound CB-839 in renal cell carcinoma cell lines.

FIG. 23, which consists of panels A-C, contains a series of bar graphs demonstrating synergy between compound CB-839 and AKT inhibitor MK2206 at various concentrations of compound CB-839 in renal cell carcinoma cell lines.

FIG. 24, which consists of panels A-C, contains a series of bar graphs demonstrating therapeutic interaction between compound CB-839 and PI3K/mTOR inhibitor BEZ235 at various concentrations of compound CB-839 in renal cell carcioma cell lines.

FIG. 25 contains a bar graph that demonstrates the therapeutic efficacy of compound CB-839, everolimus, and the combination of CB-839 and everolimus in renal clear cell carcinoma cells. The combination therapy yields synergistic anti-proliferative activity in these cancer cells.

FIG. 26 shows a bar graph demonstrating the therapeutic efficacy of compound CB-839, erlotinib, and the combination of CB-839 and erlotinib non-small cell lung cancer (NSCLC) cell lines. H1650 NSCLC cell lines provide an erlotinib-resistant model. Though these cancer cells are resistant to erlotinib, the combination of CB-839 and erlotinib enhances the anti-tumor effects of each individual compound. FIG. 26 also provides a graph showing the results of an *in vivo* xenograft study of the combination of CB-839 and erlotinib in lung cancer tumors.

Detailed Description of the Invention

The present invention provides a method of treating or preventing cancer, a myeloproliferative disease, an immunological disease, or a neurological disease comprising conjointly administering a glutaminase inhibitor and an anticancer agent selected from an enzyme inhibitor (such as a kinase inhibitor), a mitotic inhibitor, a DNA-modifying agent, and a cytidine analog.

In certain embodiments, conjointly administering the anticancer agent and glutaminase inhibitor provides improved efficacy relative to individual administration of the anticancer agent or glutaminase inhibitor as a single agent.

In certain embodiments, the conjoint administration of the anticancer agent and glutaminase inhibitor provides an additive effect.

In certain embodiments, the conjoint administration of the anticancer agent and glutaminase inhibitor provides a synergistic effect.

In certain embodiments, the anticancer agent and glutaminase inhibitor are administered simultaneously.

In certain embodiments, the anticancer agent is administered within about 5 minutes to within about 168 hours prior or after of the glutaminase inhibitor.

In certain embodiments, the anticancer 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, DNA cross-linking agents, DNA intercalating agents, and cytidine analogs.

In certain embodiments, the anticancer 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.

In certain embodiments, the anticancer agent is a PARP inhibitor.

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

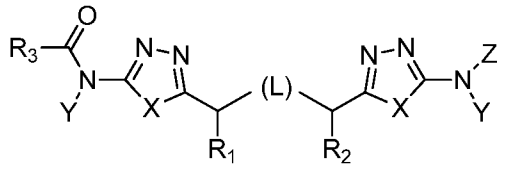
In certain embodiments, the anticancer agent is selected from vincristine, carboplatin, cisplatin, gemcitabine, MK2206, everolimus, trametinib, sunitinib,

sorafenib, BEZ235, paclitaxel, docetaxel, erlotinib, selumetinib, sirolimus, trametinib, temsirolimus, pazopanib, olaparib, and GSK1120212.

In certain embodiments, the anti-cancer agent is olaparib.

In certain embodiments, the anticancer agent is azacitidine.

In certain embodiments of the invention, 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, heterocycloxy, 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., -alkylC(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.

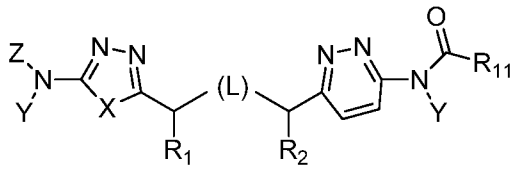
In certain embodiments wherein L represents CH_2SCH_2, X represents S, and Z represents $R_3(CO)$, both R_3 groups are not aryl, such as optionally substituted phenyl, aralkyl, such as benzyl, heteroaryl, such as 2-furyl, 2-thienyl or 1,2,4-trizole, substituted or unsubstituted alkyl, such as methyl, 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.

The present invention further provides a method of treating or preventing cancer, myeloproliferative disease or immune-related diseases comprising conjointly administering an anticancer agent and a glutaminase inhibitor, wherein the glutaminase inhibitor comprises 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.

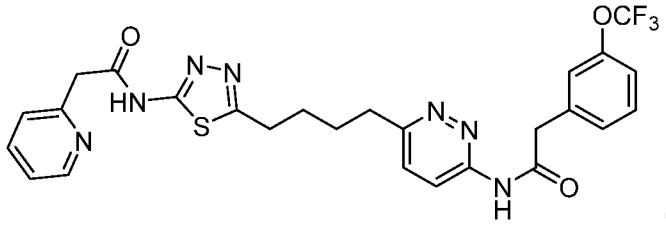
In certain embodiments, L represents CH_2SCH_2, CH_2CH_2, CH_2S or SCH_2, such as CH_2CH_2, Y represents H, X represents S, Z represents $R_3(CO)$, R_1 and R_2 each represent H, and R_3 represents $C(R_8)(R_9)(R_{10})$, wherein R_8 represents aryl, arylalkyl, heteroaryl or heteroaralkyl, such as aryl, 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 of the methods described herein, the glutaminase inhibitor is a compound having the structure of Formula (II):



(II), or a pharmaceutically acceptable salt thereof. The compound of Formula (II) is alternatively referred to herein as "CB-839."

In certain embodiments, the cancer is Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML), Adrenocortical Carcinoma, Anal Cancer, Appendix Cancer, Atypical Teratoid/Rhabdoid Tumor, Basal Cell Carcinoma, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Brain Tumor, Astrocytoma, Brain and Spinal Cord Tumor, Brain Stem Glioma, Central Nervous System Atypical Teratoid/Rhabdoid Tumor, Central Nervous System Embryonal Tumors, Breast Cancer, Bronchial Tumors, Burkitt Lymphoma, Carcinoid Tumor, Carcinoma of Unknown Primary, Central Nervous System Cancer, Cervical Cancer, Childhood Cancers, Chordoma, Chronic Lymphocytic Leukemia (CLL), Chronic Myelogenous Leukemia (CML), Chronic Myeloproliferative Disorders, Colon Cancer, Colorectal Cancer, Craniopharyngioma, Cutaneous T-Cell Lymphoma, Ductal Carcinoma In Situ (DCIS), Embryonal Tumors, Endometrial Cancer, Ependymoblastoma, Ependymoma, Esophageal Cancer, Esthesioneuroblastoma, Ewing Sarcoma, Extracranial Germ Cell Tumor, Extragonadal Germ Cell Tumor, Extrahepatic Bile Duct Cancer, Eye Cancer, Fibrous Histiocytoma of Bone, Gallbladder Cancer, Gastric Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Stromal Tumors (GIST), Germ Cell Tumor, Ovarian Germ Cell Tumor, Gestational Trophoblastic Tumor, Glioma, Hairy Cell Leukemia, Head and Neck Cancer, Heart Cancer, Hepatocellular Cancer, Histiocytosis, Langerhans Cell Cancer, Hodgkin Lymphoma, Hypopharyngeal Cancer, Intraocular Melanoma, Islet Cell Tumors, Kaposi Sarcoma, Kidney Cancer, Langerhans Cell Histiocytosis, Laryngeal Cancer, Leukemia, Lip and Oral Cavity Cancer, Liver Cancer, Lobular Carcinoma In Situ (LCIS), Lung Cancer, Lymphoma, AIDS-Related Lymphoma, Macroglobulinemia, Male Breast Cancer,

Medulloblastoma, Medulloepithelioma, Melanoma, Merkel Cell Carcinoma, Malignant Mesothelioma, Metastatic Squamous Neck Cancer with Occult Primary, Midline Tract Carcinoma Involving *NUT* Gene, Mouth Cancer, Multiple Endocrine Neoplasia Syndrome, Multiple Myeloma/Plasma Cell Neoplasm, Mycosis Fungoides, Myelodysplastic Syndrome, Myelodysplastic/Myeloproliferative Neoplasm, Chronic Myelogenous Leukemia (CML), Acute Myeloid Leukemia (AML), Myeloma, Multiple Myeloma, Chronic Myeloproliferative Disorder, Nasal Cavity Cancer, Paranasal Sinus Cancer, Nasopharyngeal Cancer, Neuroblastoma, Non-Hodgkin Lymphoma, Non-Small Cell Lung Cancer, Oral Cancer, Oral Cavity Cancer, Lip Cancer, Oropharyngeal Cancer, Osteosarcoma, Ovarian Cancer, Pancreatic Cancer, Papillomatosis, Paraganglioma, Paranasal Sinus Cancer, Nasal Cavity Cancer, Parathyroid Cancer, Penile Cancer, Pharyngeal Cancer, Pheochromocytoma, Pineal Parenchymal Tumors of Intermediate Differentiation, Pineoblastoma, Pituitary Tumor, Plasma Cell Neoplasm, Pleuropulmonary Blastoma, Breast Cancer, Primary Central Nervous System (CNS) Lymphoma, Prostate Cancer, Rectal Cancer, Renal Cell Cancer, Clear cell renal cell carcinoma, Renal Pelvis Cancer, Ureter Cancer, Transitional Cell Cancer, Retinoblastoma, Rhabdomyosarcoma, Salivary Gland Cancer, Sarcoma, Sézary Syndrome, Skin Cancer, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Cell Carcinoma, Squamous Neck Cancer with Occult Primary, Squamous Cell Carcinoma of the Head and Neck (HNSCC), Stomach Cancer, Supratentorial Primitive Neuroectodermal Tumors, T-Cell Lymphoma, Testicular Cancer, Throat Cancer, Thymoma, Thymic Carcinoma, Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, Triple Negative Breast Cancer (TNBC), Gestational Trophoblastic Tumor, Unknown Primary, Unusual Cancer of Childhood, Urethral Cancer, Uterine Cancer, Uterine Sarcoma, Waldenström Macroglobulinemia, or Wilms Tumor.

In certain embodiments, the cancer is selected from biliary cancer, breast cancer, colorectal cancer, leukemia, acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), hairy cell leukemia, T-cell leukemia, brain malignancy, lymphoma, diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, Hodgkin's lymphoma, MALT lymphoma, mantle cell lymphoma (MCL), no-Hodgkin lymphoma (NHL), endometrial cancer, head and neck cancers, Kaposi's sarcoma, lung cancer, melanoma, multiple myeloma (MM), myelodisplastic disease

(MDS), ocular disease, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, thyroid cancer, tuberous sclerosis, and Waldenstrom macrogloulinemia (WM).

In certain embodiments, the cancer is multiple myeloma.

In certain embodiments, the cancer is renal cell carcinoma.

In certain embodiments, the cancer is non-small cell lung cancer (NSCLC).

In certain embodiments, the myeloproliferative disease is selected from chronic eosinophilic leukemia, chronic myelogenous leukemia (CML), chronic neutrophilic leukemia, essential thrombocythemia, polycythemia vera, and myelofibrosis.

In certain embodiments the immunological disease is selected from ankylosing spondylitis, Crohn's disease, erythema nodosum leprosum (ENL), graft versus host disease (GVHD), HIV-associated wasting syndrome, lupus erythematosus, organ transplant rejection, post-polycythemia, psoriasis, psoriatic arthritis, recurrent aphthous ulcers, rheumatoid arthritis (RA), severe recurrent aphthous stomatitis, systemic sclerosis, and tuberous sclerosis.

In certain embodiments of the methods of the invention described herein, the anticancer 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, DNA cross-linking agents, DNA intercalating agents, and cytidine analogs.

In certain embodiments of the methods of the invention described herein, the anticancer 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.

In certain embodiments, the anticancer agent is a PARP inhibitor. Exemplary PARP inhibitors include olaparib, talazoparib, rubcaparib, and veliparib.

In certain embodiments of the methods described herein, the anticancer agent is a cytidine analog. An exemplary cytidine analog is azacitidine.

In further embodiments of the methods of the invention described herein, the anticancer agent is selected from vincristine, carboplatin, cisplatin, gemcitabine, MK2206, everolimus, trametinib, sunitinib, sorafenib, BEZ235, paclitaxel, docetaxel, erlotinib, selumetinib, sirolimus, trametinib, temsirolimus, pazopanib, and GSK1120212.

In further embodiments of the methods of the invention described herein, the anticancer 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.

In further embodiments of the methods of the invention described herein, the anticancer agent is azacitidine.

In certain embodiments, the cancer is resistant to an anticancer agent comprising an enzyme inhibitor (such as a kinase inhibitor), a mitotic inhibitor, a DNA-modifying agent, or a cytidine analog. In certain embodiments, the anticancer 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. In certain other embodiments, the cancer is resistant to an anticancer agent selected from vincristine, carboplatin, cisplatin, gemcitabine, MK2206, everolimus, trametinib, sunitinib, sorafenib, BEZ235, paclitaxel, docetaxel, erlotinib, selumetinib, sirolimus, trametinib, temsirolimus, pazopanib, olaparib, and GSK1120212. In certain embodiments, the cancer is resistant to a cytidine analog such as azacitidine.

In certain embodiments, the resistant cancer is multiple myeloma.

In certain embodiments, the resistant cancer is non-small cell lung cancer (NSCLC). In certain such embodiments, the NSCLC is resistant to erlotinib.

In certain embodiments, the present invention provides methods of treating a cancer resistant to an anticancer agent. In exemplary embodiments, the cancer is resistant to erlotinib, and the methods of treating the cancer include administration of a glutaminase inhibitor, e.g., CB-839, in combination with erlotinib as the anti-cancer agent. In certain such embodiments, the cancer is non-small cell lung cancer. In certain such embodiments, CB-839 enhances the anti-tumor effect of erlotinib.

In certain embodiments, the myeloproliferative disease is resistant to an anticancer agent selected from an enzyme inhibitor (such as a kinase inhibitor), a mitotic inhibitor, a DNA-modifying agent, and a cytidine analog. In certain embodiments, the anticancer 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. In certain other embodiments, the myeloproliferative disease is resistant to an anticancer agent selected from vincristine, carboplatin, cisplatin, gemcitabine, MK2206, everolimus, trametinib, sunitinib, sorafenib, BEZ235, paclitaxel, docetaxel, erlotinib, selumetinib, sirolimus, trametinib, temsirolimus, pazopanib, olaparib, and GSK1120212. In certain embodiments, the myeloproliferative disease is resistant to a cytidine analog such as azacitidine.

In certain embodiments, the immunological disease is resistant to an anticancer agent comprising an enzyme inhibitor (such as a kinase inhibitor), a mitotic inhibitor, a DNA-modifying agent, and a cytidine analog. In certain embodiments, the anticancer 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. In certain other embodiments, the immunological disease is resistant to an anticancer agent selected from vincristine, carboplatin, cisplatin, gemcitabine, MK2206, everolimus, trametinib, sunitinib, sorafenib, BEZ235, paclitaxel, docetaxel, erlotinib, selumetinib, sirolimus, trametinib, temsirolimus, pazopanib, olaparib, and GSK1120212. In certain embodiments, the immunological disease is resistant to a cytidine analog such as azacitidine.

In certain embodiments, the glutaminase inhibitor is selected from any one of the compounds disclosed in Table 3 of PCT Application Publication Number WO 2013/078123, published May 30, 2013, the contents of which are incorporated herein by reference. Preferably, the compound is selected from compound 1, 2, 6, 7, 8, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 35, 36, 38, 39, 40, 41, 43, 44, 47, 48, 50, 51, 52, 54, 55, 58, 63, 64, 65, 67, 68, 69, 70, 71, 72, 73, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 92, 93, 94, 95, 97, 99, 100, 102, 105, 107, 111, 112, 114, 115, 116, 117, 118, 120, 121, 122, 123, 126, 127, 133, 135, 136, 138, 140, 141, 143, 146, 147, 148, 152, 153, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 168, 169, 170, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 185, 186, 187, 188, 189, 190, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 208, 210, 211, 213, 214, 216, 217, 219, 220, 226, 227, 228, 229, 231, 232, 234, 235, 236, 237, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270,

271, 273, 274, 275, 276, 278, 279, 280, 281, 282, 283, 285, 286, 287, 288, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 302, 304, 1038, 306, 307, 308, 309, 310, 311, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 327, 329, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 527, 347, 348, 349, 350, 351, 352, 353, 354, 355, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 638, 639, 640, 641, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 707, 708, 709, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, or 730.

In certain embodiments, the glutaminase inhibitor may be a prodrug of a compound 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, glutaminase inhibitor compounds of the invention may be racemic. In certain embodiments, glutaminase inhibitor 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 cancer, such as biliary cancer, breast cancer, colorectal cancer, leukemia, acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), hairy cell leukemia, T-cell leukemia, brain malignancy, lymphoma, diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, Hodgkin's lymphoma, MALT lymphoma, mantle cell lymphoma (MCL), no-Hodgkin lymphoma (NHL), endometrial cancer, head and neck cancers, Kaposi's sarcoma, lung cancer, melanoma, multiple myeloma (MM), myelodisplastic disease (MDS), ocular disease, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, renal cell carcinoma, thyroid cancer, tuberous sclerosis, and Waldenstrom macrogloulinemia (WM), with an anticancer agent, such as vincristine, carboplatin, cisplatin, gemcitabine, MK2206, everolimus, trametinib, sunitinib, sorafenib, BEZ235, paclitaxel, docetaxel, erlotinib, selumetinib, sirolimus, trametinib, temsirolimus, pazopanib, or GSK1120212, and a glutaminase inhibitor, such as a compound of formula I, Ia, II, or a pharmaceutically acceptable salt thereof.

In certain embodiments, the present invention relates to methods of treating or preventing cancer with an anticancer agent such as azacitidine and a glutaminase inhibitor, such as a compound of formula I, Ia, II, or a pharmaceutically acceptable salt thereof.

In certain exemplary embodiments, the present invention provides methods of treating cancer with a glutaminase inhibitor, e.g., CB-839, in combination with everolimus as the anti-cancer agent. In certain such embodiments, the cancer is renal cell carcinoma. In certain such embodiments, the combination of CB-839 and everolimus in cancer therapies provides a synergistic effect.

In certain embodiments, the present invention relates to methods of treating or preventing myeloproliferative disease, such as chronic eosinophilic leukemia, chronic myelogenous leukemia (CML), chronic neutrophilic leukemia, essential

thrombocythemia, polycythemia vera, and myelofibrosis, with an anticancer agent, such as vincristine, carboplatin, cisplatin, gemcitabine, MK2206, everolimus, trametinib, sunitinib, sorafenib, BEZ235, paclitaxel, docetaxel, erlotinib, selumetinib, sirolimus, trametinib, temsirolimus, pazopanib, olaparib, or GSK1120212, and a glutaminase inhibitor, such as a compound of formula I, Ia, II, or a pharmaceutically acceptable salt thereof. In certain embodiments, the methods of treating or preventing a myeloproliferative disease comprise administration of an anticancer agent, such as azacitidine, and a glutaminase inhibitor, such as a compound of formula I, Ia, II, or a pharmaceutically acceptable salt thereof.

In certain embodiments, the present invention relates to methods of treating or preventing immune-related disease, such as ankylosing spondylitis, Crohn's disease, erythema nodosum leprosum (ENL), graft versus host disease (GVHD), HIV-associated wasting syndrome, lupus erythematosus, organ transplant rejection, post-polycythemia, psoriasis, psoriatic arthritis, recurrent aphthous ulcers, rheumatoid arthritis (RA), severe recurrent aphthous stomatitis, systemic sclerosis, and tuberous sclerosis, with an anticancer agent, such as vincristine, carboplatin, cisplatin, gemcitabine, MK2206, everolimus, trametinib, sunitinib, sorafenib, BEZ235, paclitaxel, docetaxel, erlotinib, selumetinib, sirolimus, trametinib, temsirolimus, pazopanib, olaparib, or GSK1120212, and a glutaminase inhibitor, such as a compound of formula I, Ia, II, or a pharmaceutically acceptable salt thereof. In certain embodiments, the methods of treating or preventing an immune-related disease comprise administration of an anticancer agent, such as azacitidine, and a glutaminase inhibitor, such as a compound of formula I, Ia, II, or a pharmaceutically acceptable salt thereof.

In certain embodiments, the present invention may be a pharmaceutical composition comprising an anticancer agent, such as vincristine, carboplatin, cisplatin, gemcitabine, MK2206, everolimus, trametinib, sunitinib, sorafenib, BEZ235, paclitaxel, docetaxel, erlotinib, selumetinib, sirolimus, trametinib, temsirolimus, pazopanib, olaparib, or GSK1120212, and a glutaminase inhibitor, such as a compound of formula I, Ia, II, or a pharmaceutically acceptable salt thereof.

In certain embodiments, the present invention provides a pharmaceutical composition comprising an anticancer agent, such as azacitidine, and a glutaminase inhibitor, such as a compound of formula I, Ia, II, or a pharmaceutically acceptable salt thereof.

In certain embodiments, the therapeutic preparation of the glutaminase inhibitor 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 glutaminase inhibitor 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 glutaminase inhibitor 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 glutaminase inhibitor 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, comprising an anticancer agent such as an enzyme inhibitor (such as a kinase inhibitor), a mitotic inhibitor, a DNA-modifying agent, or a cytidine analog and any of the compounds shown above (e.g., a glutaminase inhibitor, such as a compound of formula I, Ia, or II), 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.

One embodiment of the present invention provides a pharmaceutical kit comprising an anticancer agent, such as vincristine, carboplatin, cisplatin, gemcitabine, MK2206, everolimus, trametinib, sunitinib, sorafenib, BEZ235, paclitaxel, docetaxel, erlotinib, selumetinib, sirolimus, trametinib, temsirolimus, pazopanib, olaparib, or GSK1120212, and a glutaminase inhibitor, such as a compound of formula I, Ia, II, or a pharmaceutically acceptable salt thereof, and directions on how to administer the anticancer agent and glutaminase inhibitor.

In other embodiments, the present invention provides a pharmaceutical kit comprising an anticancer agent, such as azacitidine, and a glutaminase inhibitor, such as a compound of formula I, Ia, II, or a pharmaceutically acceptable salt thereof, and directions on how to administer the anticancer agent and glutaminase inhibitor.

Uses of the Invention

Combination therapy is an important treatment modality in many disease settings, such as cancer. Recent scientific advances have increased our understanding of the pathophysiological processes that underlie these and other complex diseases. This increased understanding has provides impetus to develop new therapeutic approaches using combinations of drugs directed at multiple therapeutic targets to improve treatment response, minimize development of resistance, or minimize adverse events. In settings in which combination therapy provides significant therapeutic advantages, there is growing interest in the development of combinations with new investigational drugs, such as glutaminase inhibitors.

Although interest in combination therapy, sometimes referred to as polytherapy, has been most prominent in oncology, it also has potential application in other therapeutic settings such as immunological diseases.

When considering the administration of multiple therapeutic agents together, one must be concerned about what sort of drug interactions will be observed. This action can be positive (when the drug's effect is increased) or antagonistic (when the drug's effect is decreased) or a new side effect can be produced that neither produces on its own.

When the interaction causes an increase in the effects of one or both of the drugs the interaction, the degree to which the final effect of the combined drugs is greater than administering either drug alone can be calculated resulting in what is called the "combination index"(CI) (Chou and Talalay, 1984). A combination index at or around 1 is considered "additive"; whereas a value greater than 1 is considered "synergistic".

The present invention provides a combination therapy comprising an anticancer agent, such as an enzyme inhibitor (such as a kinase inhibitor), a mitotic inhibitor), a DNA-modifying agent, or a cytidine analog, and a glutaminase inhibitor. In certain embodiments, the combination therapy treats or prevents cancer, a myeloproliferative disorder, an immunological disorder, or a neurological disorder.

Certain embodiments of the invention relate to treating cancer comprising administering an anticancer agent and a glutaminase inhibitor. In certain embodiments, the cancer may be one or a variant of a cancer selected from Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML), Adrenocortical Carcinoma, Anal Cancer, Appendix Cancer, Atypical Teratoid/Rhabdoid Tumor, Basal Cell Carcinoma, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Brain Tumor, Astrocytoma, Brain and Spinal Cord Tumor, Brain Stem Glioma, Central Nervous System Atypical Teratoid/Rhabdoid Tumor, Central Nervous System Embryonal Tumors, Breast Cancer, Bronchial Tumors, Burkitt Lymphoma, Carcinoid Tumor, Carcinoma of Unknown Primary, Central Nervous System Cancer, Cervical Cancer, Childhood Cancers, Chordoma, Chronic Lymphocytic Leukemia (CLL), Chronic Myelogenous Leukemia (CML), Chronic Myeloproliferative Disorders, Colon Cancer, Colorectal Cancer, Craniopharyngioma, Cutaneous T-Cell Lymphoma Ductal Carcinoma In Situ (DCIS), Embryonal Tumors, Endometrial Cancer, Ependymoblastoma, Ependymoma, Esophageal Cancer, Esthesioneuroblastoma, Ewing Sarcoma, Extracranial Germ Cell Tumor, Extragonadal Germ Cell Tumor, Extrahepatic Bile Duct Cancer, Eye Cancer, Fibrous Histiocytoma of Bone, Gallbladder Cancer, Gastric Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Stromal Tumors (GIST), Germ Cell Tumor, Extracranial Germ Cell Tumor, Extragonadal Germ Cell Tumor, Ovarian Germ Cell Tumor, Gestational Trophoblastic Tumor, Glioma, Hairy Cell Leukemia, Head and Neck Cancer, Heart Cancer, Hepatocellular Cancer, Histiocytosis, Langerhans Cell Cancer, Hodgkin Lymphoma, Hypopharyngeal Cancer, Intraocular Melanoma, Islet Cell Tumors, Kaposi Sarcoma, Kidney Cancer, Langerhans Cell Histiocytosis, Laryngeal Cancer, Leukemia, Lip and Oral Cavity Cancer, Liver Cancer, Lobular Carcinoma In Situ (LCIS), Lung Cancer, Lymphoma, AIDS-Related Lymphoma, Macroglobulinemia, Male Breast Cancer, Medulloblastoma, Medulloepithelioma, Melanoma, Merkel Cell Carcinoma, Malignant Mesothelioma, Metastatic Squamous Neck Cancer with Occult Primary, Midline Tract Carcinoma Involving *NUT* Gene, Mouth Cancer, Multiple Endocrine Neoplasia Syndrome, Multiple Myeloma/Plasma Cell Neoplasm, Mycosis Fungoides, Myelodysplastic Syndrome, Myelodysplastic/Myeloproliferative Neoplasm, Chronic Myelogenous Leukemia (CML), Acute Myeloid Leukemia (AML), Myeloma, Multiple Myeloma, Chronic Myeloproliferative Disorder, Nasal Cavity Cancer, Paranasal Sinus Cancer, Nasopharyngeal Cancer, Neuroblastoma,

Non-Hodgkin Lymphoma, Non-Small Cell Lung Cancer, Oral Cancer, Oral Cavity Cancer, Lip Cancer, Oropharyngeal Cancer, Osteosarcoma, Ovarian Cancer, Pancreatic Cancer, Papillomatosis, Paraganglioma, Paranasal Sinus Cancer, Nasal Cavity Cancer, Parathyroid Cancer, Penile Cancer, Pharyngeal Cancer, Pheochromocytoma, Pineal Parenchymal Tumors of Intermediate Differentiation, Pineoblastoma, Pituitary Tumor, Plasma Cell Neoplasm, Pleuropulmonary Blastoma, Breast Cancer, Primary Central Nervous System (CNS) Lymphoma, Prostate Cancer, Rectal Cancer, Renal Cell Cancer, Clear cell renal cell carcinoma, Renal Pelvis Cancer, Ureter Cancer, Transitional Cell Cancer, Retinoblastoma, Rhabdomyosarcoma, Salivary Gland Cancer, Sarcoma, Sézary Syndrome, Skin Cancer, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Cell Carcinoma, Squamous Neck Cancer with Occult Primary (e.g., Metastatic), Squamous Cell Carcinoma of the Head and Neck (HNSCC), Stomach Cancer, Supratentorial Primitive Neuroectodermal Tumors, T-Cell Lymphoma, Testicular Cancer, Throat Cancer, Thymoma, Thymic Carcinoma, Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, Triple Negative Breast Cancer (TNBC), Gestational Trophoblastic Tumor, Unknown Primary, Unusual Cancer of Childhood, Urethral Cancer, Uterine Cancer, Uterine Sarcoma, Waldenström Macroglobulinemia, and Wilms Tumor.

In certain embodiments the cancer is selected from biliary cancer, breast cancer, colorectal cancer, leukemia, acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), hairy cell leukemia, T-cell leukemia, brain malignancy, lymphoma, diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, Hodgkin's lymphoma, MALT lymphoma, mantle cell lymphoma (MCL), no-Hodgkin lymphoma (NHL), endometrial cancer, head and neck cancers, Kaposi's sarcoma, lung cancer, melanoma, multiple myeloma (MM), myelodisplastic disease (MDS), ocular disease, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, thyroid cancer, tuberous sclerosis, and Waldenstrom macrogloulinemia (WM).

Myeloproliferative disorders (also refered to as myeloproliferative diseases) are a type of disease in which the bone marrow makes too many red blood cells, platelets, or certain white blood cells. Myeloproliferative disorders usually get worse over time as the number of extra cells build up in the blood and/or bone marrow. This may cause bleeding problems, anemia, infection, fatigue, or other signs and

symptoms. Certain myeloproliferative disorders may become acute myeloid leukemia (AML). Myeloproliferative disorders include chronic myelogenous leukemia (CML), polycythemia vera, primary myelofibrosis, essential thrombocythemia, chronic neutrophilic leukemia, and chronic eosinophilic leukemia. In certain embodiments, the invention comprises treating a myeloproliferative disorder comprising administering an anticancer agent as described herein and a glutaminase inhibitor.

Diseases involving the immune system (referred to as immunological, immune-mediated or immune-related disorders/diseases) are caused by abnormally low activity or overactivity of the immune system. In cases of immune system overactivity, the body attacks and damages its own tissues (also known as autoimmune disease). Although not all-inclusive, some known immune-related disorders include ankylosing spondylitis, Crohn's disease, erythema nodosum leprosum (ENL), graft versus host disease (GVHD), HIV-associated wasting syndrome, lupus erythematosus, organ transplant rejection, post-polycythemia, psoriasis, psoriatic arthritis, recurrent aphthous ulcers, rheumatoid arthritis (RA), severe recurrent aphthous stomatitis, systemic sclerosis, and tuberous sclerosis. In certain embodiments, the invention provides for the method of treating an immune-related disease comprising administering an anticancer agent and a glutaminase inhibitor. In certain embodiments the immune-mediated disorder is selected from ankylosing spondylitis, Crohn's disease, erythema nodosum leprosum (ENL), graft versus host disease (GVHD), HIV-associated wasting syndrome, lupus erythematosus, organ transplant rejection, post-polycythemia, psoriasis, psoriatic arthritis, recurrent aphthous ulcers, rheumatoid arthritis (RA), severe recurrent aphthous stomatitis, systemic sclerosis, and tuberous sclerosis.

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. The conversion of glutamine into glutamate is initated by the mitochondrial enzyme, glutaminase ("GLS"). 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, 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 been identified recently and has similar activity characteristics of GLS1. In certain embodiments, the glutaminase inhibitor compounds may selectively inhibit GLS1, GLS2 and GAC. In a preferred embodiment, the glutaminase inhibitor compounds selectively inhibit GLS1 and GAC.

In one embodiment, the method of treating or preventing cancer, myeloproliferative disorder or immune-related disease may further comprise administering one or more additional therapeutic agents conjointly with the anticancer agent and glutaminase inhibitor. Therapeutic agents that may be conjointly administered with compounds of the invention include: aminoglutethimide, amsacrine, anastrozole, asparaginase, 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, erlotinib, 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, miltefosine, mitomycin, mitotane, mitoxantrone, MK-2206, nilutamide, nocodazole, octreotide, olaparib, oxaliplatin, paclitaxel, pamidronate, pazopanib, pentostatin, perifosine, plicamycin, pomalidomide, porfimer, procarbazine, raltitrexed, rituximab, 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, and vinorelbine.

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
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

Name	Therapeutic agents
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, 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	Chlorambucil, Prednisone

Name	Therapeutic agents
lymphocytic leukemia)	
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
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

Name	Therapeutic agents
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
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

Name	Therapeutic agents
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
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

Name	Therapeutic agents
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
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

Name	Therapeutic agents
7 + 3	Cytarabine with/, Daunorubicin or Idarubicin or Mitoxantrone
"8 in 1"	Methylprednisolone, Vincristine, Lomustine, Procarbazine, Hydroxyurea, Cisplatin, Cytarabine, Dacarbazine

Cellular pathways operate more like webs than superhighways. There are multiple redundancies, or alternate routes, that may be activated in response to the inhibition of a pathway. This redundancy promotes the emergence of resistant cells or organisms under the selective pressure of a targeted agent, resulting in drug resistance and clinical relapse.

In some cases, one can overcome the resistance by the addition of another therapeutic agent. As demonstrated in FIGs. 1-5, treatment of multiple myeloma cells with both an anticancer agent (e.g., vincristine, MK2206, everolimus, trametinib, sorafenib) and a glutaminase inhibitor resulted in a synergistic effect. For this reason, combination therapies are often needed to effectively treat many tumors and immunological diseases.

In certain embodiments of the invention, the anticancer agent is administered simultaneously with the glutaminase inhibitor. In certain embodiments, the anticancer agent is administered within about 5 minutes to within about 168 hours prior or after of the glutaminase inhibitor.

In certain embodiments, the present invention provides a kit comprising: a) an anticancer agent; b) a glutaminase inhibitor; and c) instructions for the administration of the compounds.

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.

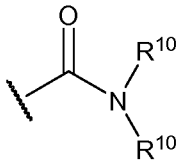
The term "C_{x-y}" when used in conjunction with a chemical moiety, such as, acyl, acyloxy, alkyl, alkenyl, alkynyl, or alkoxy is meant to include groups that contain from x to y carbons in the chain. For example, the term "C_{x-y}alkyl" refers to substituted or unsubstituted saturated hydrocarbon groups, 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-trifluoroethyl, 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-.

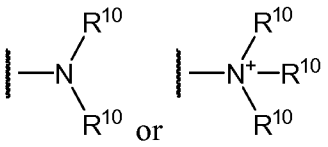
The term "alkynyl", as used herein, refers to an aliphatic group containing at least one triple bond and is intended to include both "unsubstituted alkynyls" and "substituted alkynyls", the latter of which refers to alkynyl moieties having substituents replacing a hydrogen on one or more carbons of the 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



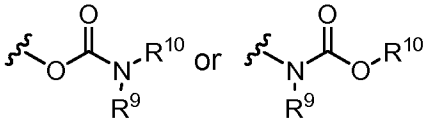
wherein each R^{10} independently represents a hydrogen or a hydrocarbyl group, or two R^{10} are taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure.

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.,

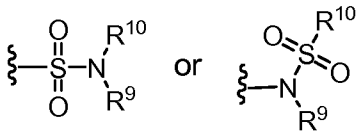
the rings are "fused rings". Each of the rings of the polycycle can be substituted or unsubstituted. In certain embodiments, each ring of the polycycle contains from 3 to 10 atoms in the ring, preferably from 5 to 7.

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.

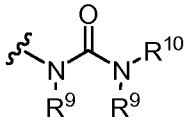
The term "sulfone" is art-recognized and refers to the group -$S(O)_2$-R^{10}, wherein R^{10} represents a hydrocarbyl.

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, 5,763,493, 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.

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

Examples

Example 1: Compound Assays

Glutaminase inhibitor compounds were assayed in both an in vitro biochemical assay and a cell proliferation assay as follows. Exemplary compounds and the IC_{50} results are provided in Table 2, below, and also in Table 3 of PCT Application Publication Number WO 2013/078123, published May 30, 2013, the contents of which are incorporated herein by reference.

Recombinant Enzyme assay

Compounds were assessed for their ability to inhibit the enzymatic activity of a recombinant form of Glutaminase 1 (GAC) using a biochemical assay that couples the production of glutamate (liberated by GAC) to glutamate dehydrogenase (GDH) and measuring the change in absorbance for the reduction of NAD^+ to NADH. Substrate solution was prepared (50 mM Tris-HCl pH 8.0, 0.2 mM EDTA, 150 mM K_2HPO_4, 0.1 mg/ml BSA, 1 mM DTT, 20mM L-glutamine, 2 mM NAD^+, and 10 ppm

antifoam) and 50 μL added to a 96-well half area clear plate (Corning #3695). Compound (2 μL) was added to give a final DMSO concentration of 2% at 2X the desired concentration of compound. Enzymatic reaction was started with the addition of 50 μL of enzyme solution (50 mM Tris-HCl pH 8.0, 0.2 mM EDTA, 150 mM K_2HPO_4, 0.1 mg/ml BSA, 1 mM DTT, 10 ppm antifoam, 4 units/ml GDH, 4 mM adenosine diphosphate, and 4 nM GAC) and read in a Molecular Devices M5 plate reader at 20 °C. The plate reader was configured to read absorbance (λ=340 nm) in kinetic mode for 15 minutes. Data was recorded as milli-absorbance units per minute and slopes were compared to a control compound and a DMSO-only control on the same plate. Compounds with slopes less than the DMSO control were considered inhibitors and plate variability was assessed using the control compound.

Results from this assay for several compounds of the invention are shown in Table 2, below, and in PCT Application Publication Number WO 2013/07812, expressed as IC_{50}, or half maximal inhibitory concentration, wherein IC_{50} is a quantitative measure indicating how much compound is needed to inhibit a given biological activity by half.

Recombinant Enzyme assay – Time Dependence

Compounds were assessed for their ability to inhibit the enzymatic activity of a recombinant form of Glutaminase 1 (GAC) using a biochemical assay that couples the production of glutamate (liberated by GAC) to glutamate dehydrogenase (GDH) and measuring the change in absorbance for the reduction of NAD^+ to NADH. Enzyme solution was prepared (50 mM Tris-HCl pH 8.0, 0.2 mM EDTA, 150 mM K_2HPO_4, 0.1 mg/ml BSA, 1 mM DTT, 10 ppm antifoam, 4 units/ml GDH, 4 mM adenosine diphosphate, and 4 nM GAC) and 50 μL added to a 96-well half area clear plate (Corning #3695). Compound (2 μL) was added to give a final DMSO concentration of 2% at 2X the desired concentration of compound. The enzyme/compound mix was sealed with sealing foil (USA Scientific) and allowed to incubate, with mild agitation, for 60 minutes at 20°C. Enzymatic reaction was started with the addition of 50 μL of substrate solution (50 mM Tris-HCl pH 8.0, 0.2 mM EDTA, 150 mM K_2HPO_4, 0.1 mg/ml BSA, 1 mM DTT, 20mM L-glutamine, 2 mM NAD^+, and 10 ppm antifoam) and read in a Molecular Devices M5 plate reader at 20°C. The plate reader was configured to read absorbance (λ=340 nm) in kinetic

mode for 15 minutes. Data was recorded as milli-absorbance units per minute and slopes were compared to a control compound and a DMSO-only control on the same plate. Compounds with slopes less than the DMSO control were considered inhibitors and plate variability was assessed using the control compound.

Results from this assay for several compounds of the invention are shown in Table 2, below, and in PCT Application Publication Number WO 2013/07812, expressed as IC_{50}, or half maximal inhibitory concentration, wherein IC_{50} is a quantitative measure indicating how much compound is needed to inhibit a given biological activity by half.

Cell proliferation assay

P493-6 (myc "on") cells were maintained in growth media (RPMI-1640, 10%FBS, 2mM glutamine, 100 units/ml Penicillin and 100μg/ml streptomycin) at 37°C with 5% CO_2. For compound assay, P493-6 cells were plated in 96-well V-bottom plates on the day of compound addition in 50 μl of growth media at a cell density of 200,000 cells/ml (10,000 cells/well). Compounds were serially diluted in 100% DMSO at 200-times the final concentration. Compounds were diluted 100-fold into growth media and then 50 μl of this mixture was added to cell plates making the final concentration of DMSO 0.5%. Cells were incubated with compound for 72 hrs at 37°C with 5% CO_2 and analyzed for antiproliferative effects either by Cell Titer Glo (Promega) or FACS analysis using the Viacount (Millipore) kit on the Guava instrument.

Results from this assay for several compounds of the invention are shown in Table 2, below, and in PCT Application Publication Number WO 2013/07812, expressed as IC_{50}, or half maximal inhibitory concentration, wherein IC_{50} is a quantitative measure indicating how much compound is needed to inhibit a given biological activity by half.

Modified Recombinant Enzyme assay – Time Dependence

Compounds were assessed for their ability to inhibit the enzymatic activity of a recombinant form of glutaminase using a biochemical assay that couples the

production of Glu (liberated by glutaminase) to GDH and measures the increase in fluorescence due to the reduction of NADP+ to NADPH.

Assay Set-up: Glutaminase reaction buffer was prepared [50 mM Tris-HCl pH 8.8, 150 mM K_2HPO_4, 0.25 mM EDTA, 0.1 mg/ml BSA (Calbiochem no. 2960), 1 mM DTT, 2 mM NADP+ (Sigma Aldrich no. N5755), and 0.01% TX-100] and used to make 3x-enzyme-containing solution, 3x-substrate-containing solution, and 3x-inhibitor-containing solution (see below). Inhibitor-containing solution was made by diluting DMSO stocks of compounds into the glutaminase reaction buffer to create a 3x inhibitor solution containing 6% DMSO. 3x-enzyme-containing solution was made by diluting recombinant glutaminase and GDH from Proteus species (Sigma Aldrich no. G4387) into glutaminase buffer to create a 6 nM glutaminase plus 18 units/mL GDH solution. A 3x substrate solution containing either Gln, Glu, or NADPH was made by diluting a stock of Gln (Sigma Aldrich no. 49419), Glu (Sigma Aldrich no. 49449), or NADPH (Sigma Aldrich no. N1630) into glutaminase reaction buffer to create a 3x-substrate solution. Reactions were assembled in a 384-well low-volume black microtiter plates (Molecular Devices no. 0200-5202) by mixing 5 μL of inhibitor-containing solution with 5 μL of substrate-containing solution followed by 5 μL of enzyme-containing solution when no preincubation was required. When time-dependent effects of compound inhibition were tested, enzyme-containing solution was treated with inhibitor-containing solution for the indicated time prior to addition of substrate-containing solution.

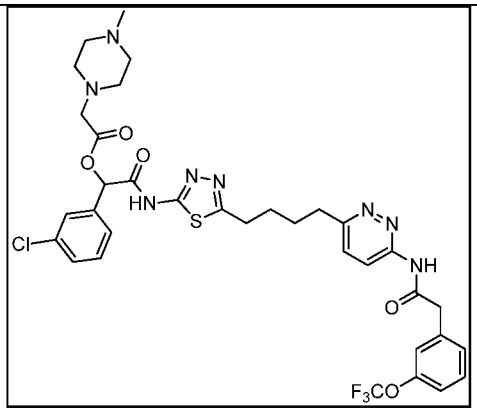
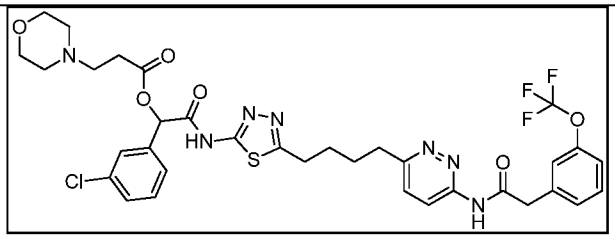
Measurement of glutaminase activity: Following the mixture of all three components, fluorescence increase (Ex: 340 nM, Em:460 nm) was recorded for 15 min at room temperature using the Spectromax M5e (Molecular Devices).

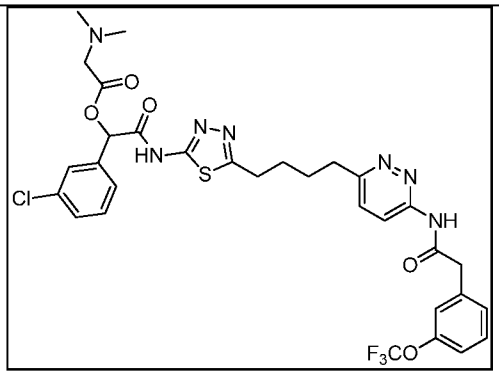
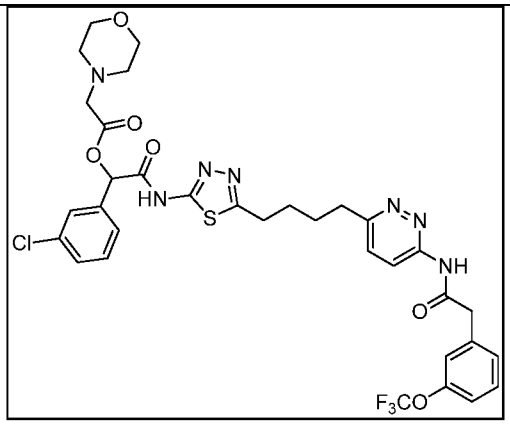
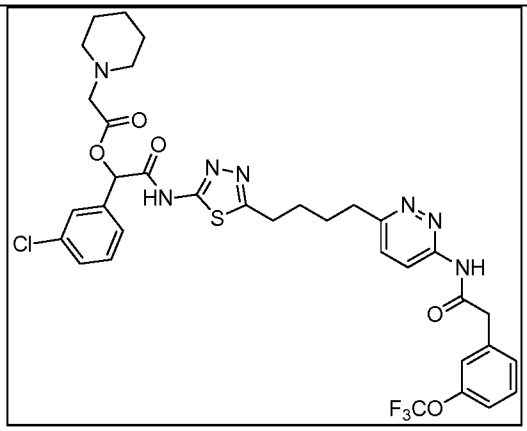
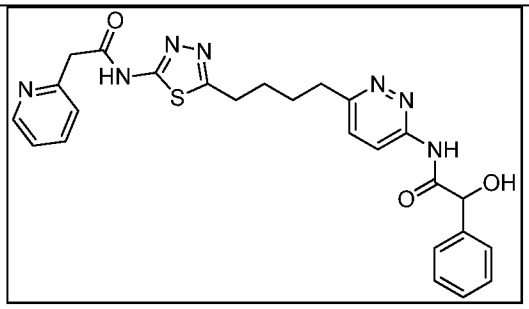
IC_{50} Determination: The initial velocities of each progress curve were calculated using a straight line equation (Y=Yintercept + (slope) * X). Initial velocity values were plotted against compound concentration and fit to a four parameter dose response equation (% activity =Bottom + (Top-Bottom)/(1+10^((LogIC_{50}-X)*HillSlope))) to calculate an IC_{50} value.

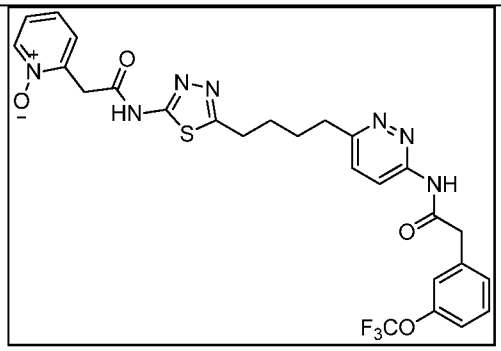
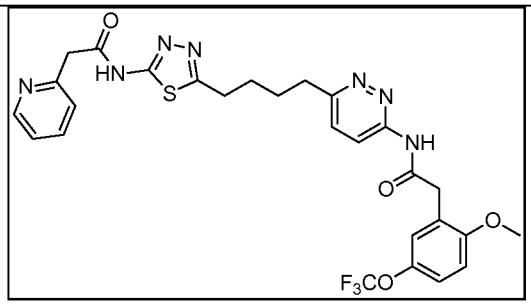
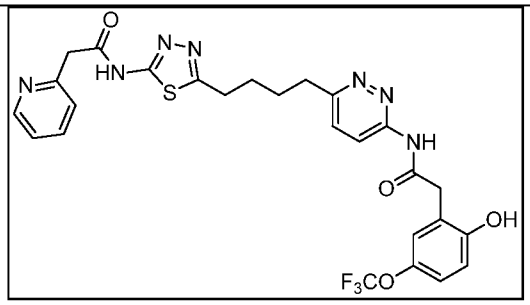
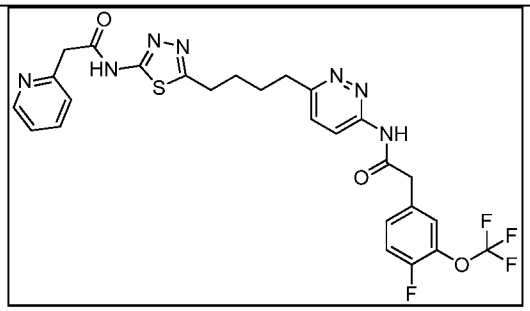
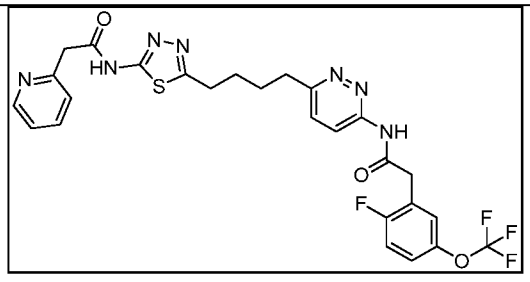
Results from this assay for several compounds are shown in Table 2, below, and in PCT Application Publication Number WO 2013/07812, expressed as IC_{50}, or

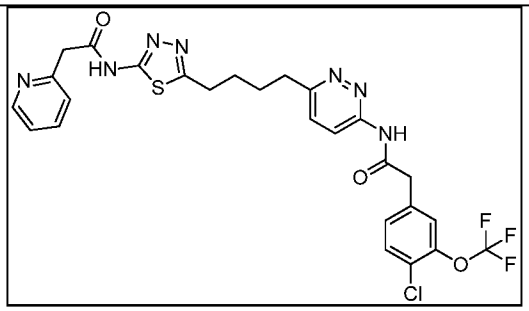
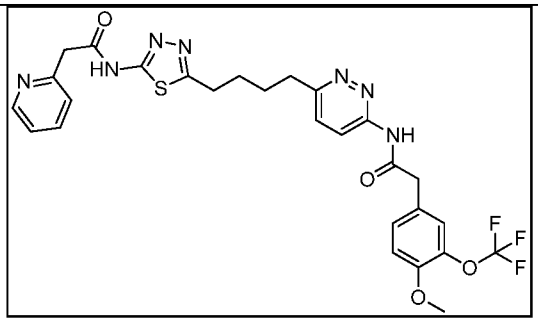
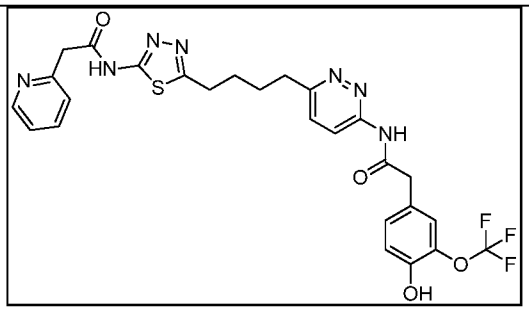
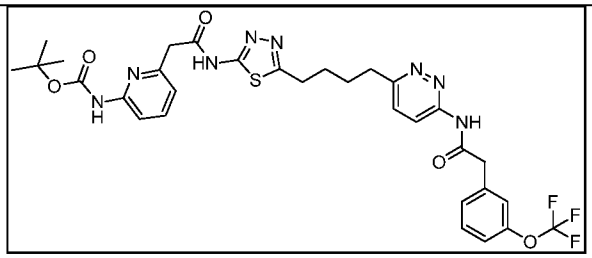
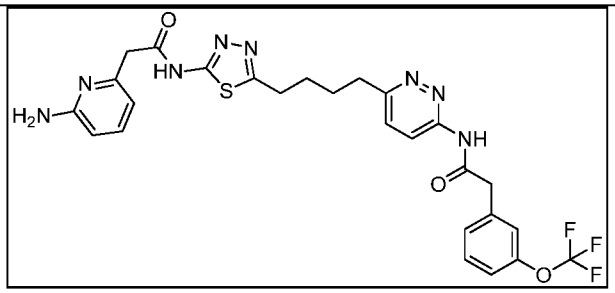
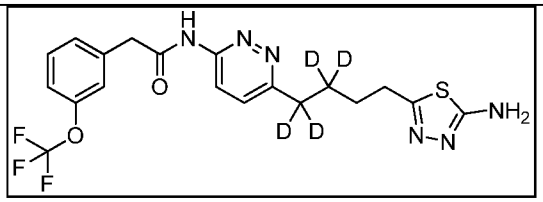
half maximal inhibitory concentration, wherein IC_{50} is a quantitative measure indicating how much compound is needed to inhibit a given biological activity by half.

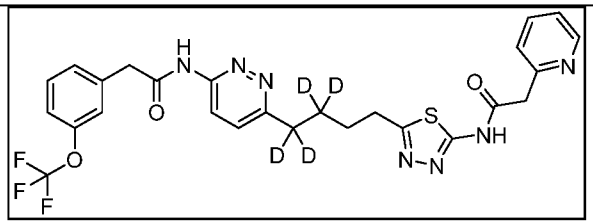
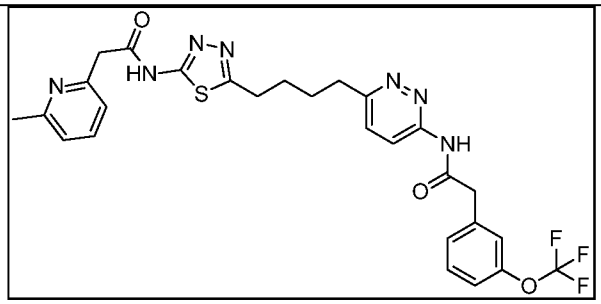
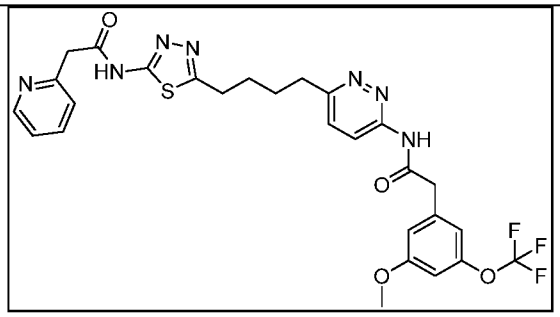
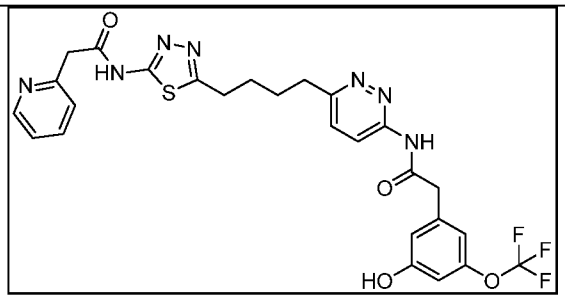
Table 2:

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

Example 2: Co-Administation of Glutaminase Inhibitor and Anti-Cancer Agent

Cells were treated with a dose titration of either CB-839, an anti-cancer agent or a mixture thereof 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). Cell proliferation for all compound treatments are represented as bar graphs, where luminescent output, Relative Light Units (RLU), correlates with viable cell number. Combination indices were calculated using the Calcusyn program (biosoft.com) and reported for individual mixtures of CB-839 and each agent.

Results for combination therapy are shown in FIGs. 1-26.

Example 3: Xenograft study with CB-839, erlotinib, and combination CB-839 and erlotinib.

Female scid/beige mice (age 7-9 weeks) were implanted subcutaneously with 1×10^7 H1650 lung cancer cells mixed 1:1 with matrigel. Tumors were measured with calipers three times per week and tumor volume calculated using the formula tumor volume (mm^3) = ($a \times b^2/2$) where 'b' is the smallest diameter and 'a' is the largest perpendicular diameter. When tumor volume had increased in three consecutive measurements (mean tumor volume ~450mm^3) mice were randomized into the following four treatment groups of n=10 mice per group: 1) Vehicle control (25% Hydroxypropyl-β-cyclodextrin) dosed orally BID; 2) CB-839 (Compound 670) at 200 mg/kg (formulated at 20 mg/mL in 25% HP-β-CD) dosed orally BID; 3) erlotinib at 1 mg/kg (formulated in 0.5% CMC/0.1% PS80) dosed orally QD; and 4) CB-839 at 200 mg/kg orally BID and erlotinib at 1 mg/kg dosed orally once daily. **P-value < 0.01 (Two-sided T-test). Results are shown in Figure 26.

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.

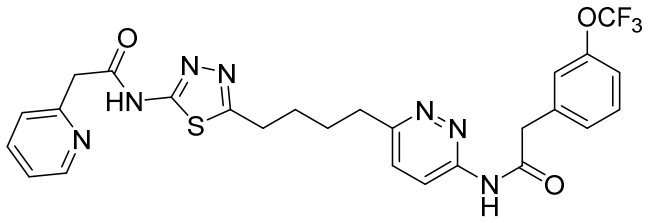
In particular, suitable compounds for practicing the invention are described in U.S. Patent No. 8,604,016, U.S. Application No. 14/081,175, and U.S. Application No. 14/095,299, which are hereby incorporated by reference herein in their entirety.

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. Use of a glutaminase inhibitor in the manufacture of a medicament for the treatment or prevention of cancer, wherein the medicament is to be conjointly administered with an anticancer agent; wherein the anti-cancer agent is everolimus;
the glutaminase inhibitor is a compound of formula II:



(II), or a pharmaceutically acceptable salt thereof; and
the cancer is renal cell carcinoma, multiple myeloma, triple negative breast cancer, or non-small cell lung cancer.

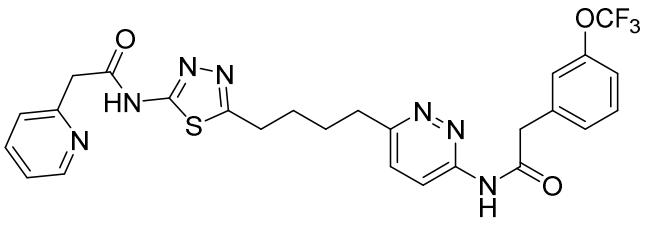
2. The use of claim 1, wherein the cancer is renal cell carcinoma.

3. The use of claim 1, wherein the cancer is multiple myeloma.

4. The use of claim 1, wherein the cancer is triple negative breast cancer.

5. The use of claim 1, wherein the cancer is non-small cell lung cancer.

6. Use of a glutaminase inhibitor in the manufacture of a medicament for the treatment or prevention of cancer, wherein the medicament is to be conjointly administered with an anticancer agent; wherein the anti-cancer agent is erlotinib;
the glutaminase inhibitor is a compound of formula II:



(II), or a pharmaceutically acceptable salt thereof; and

the cancer is non-small cell lung cancer.

7. The use of claim 6, wherein the non-small cell lung cancer is resistant to erlotinib.