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COMBINATION THERAPY WITH A MUTANT IDH INHIBITOR AND A BCL-2 INHIBITOR

The present invention relates to combination therapy with a mutant isocitrate
5 dehydrogenase (IDH) inhibitor and Bcl-2 inhibitor, for the treatment of cancer.

IDH and IDH2 are enzymes that catalyze the conversion of isocitrate to α -ketoglutarate (α -KG), and reduces nicotinamide adenine dinucleotide phosphate (NADP⁺) to NADPH (Megias-Vericat J, *et al.*, *Blood Lymph. Cancer: Targets and Therapy* 2019; 9: 19-32).

10 Neomorphic (*de novo*) mutations in IDH1, *e.g.*, at IDH1 amino acid residue R132, contribute to tumorigenesis in several types of cancer, including solid tumor cancers and hematologic malignancies (Badur MG, *et al.*, *Cell Reports* 2018; 25: 1680). IDH1 mutations can result in high levels of 2-hydroxyglutarate (2-HG), which inhibits cellular differentiation, and inhibitors of mutant IDH1 can reduce 2-HG levels, which promotes
15 cellular differentiation (Molenaar RJ, *et al.*, *Oncogene* 2018; 37: 1949-1960). Mutations also occur in IDH2, *e.g.*, at amino acid residues R172, R140 and R172 (Yang H, *et al.*, *Clin. Cancer. Res.* 2012; 18: 5562-5571; Mondesir J, *et al.*, *J. Blood Med.* 2016; 7: 171-180).

For example, acute myeloid leukemia (AML) is characterized by a diverse
20 spectrum of mutated genes and a multi-clonal genomic architecture comprising preleukemic and leukemic clones that evolve dynamically over time and under the selective pressure of therapy (Bloomfield CD, *et al.*, *Blood Revs.* 2018; 32: 416-425). Induction chemotherapy with cytarabine and an anthracycline ("7 + 3") has been the standard of care for more than 4 decades for subjects with newly diagnosed AML.

25 In recent years, five additional drugs have been approved by the U.S. Food and Drug Administration for treating AML: midostaurin, enasidenib, CPX-351, gemtuzumab ozogamicin (Bloomfield CD, *et al.*, *Blood Revs.* 2018; 32: 416-425), and ivosidenib (Megias-Vericat J, *et al.*, *Blood Lymph. Cancer: Targets and Therapy* 2019; 9: 19-32). Approximately 60% to 70% of adults with AML can be expected to attain complete
30 remission (CR) status following appropriate induction therapy, and more than 25% of

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adults with AML (about 45% of those who attain CR) can be expected to survive 3 or more years and may be cured.

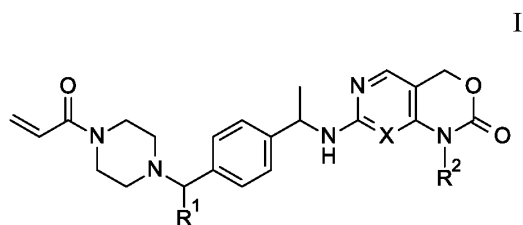
However, IDH1 resistance mutations are observed in 7–14% of AML subjects, and the associated high 2-HG level can result in an epigenetic hyper-methylation phenotype and a block in differentiation, resulting in leukemogenesis (Megias-Vericat J, *et al.*, *Blood Lymph. Cancer: Targets and Therapy* 2019; 9: 19-3). In addition, mutations in the Flt3 kinase are observed in approximately one third of AML subjects (Lee HJ, *et al.*, *Oncotarget* 2018; 9: 924-936).

Thus, there remains a need for alternative mutant IDH-related cancer therapies.

Certain mutant IDH1 and IDH2 inhibitors are disclosed in WO 2018/111707 A1, including a compound defined herein as “Compound A,” which is a covalent inhibitor of mutant IDH1 that modifies a single cysteine (Cys269) in an allosteric binding pocket, rapidly inactivates the enzyme, and selectively inhibits 2-HG production, without affecting α -KG levels (WO 2018/111707 A1).

The present invention provides a method of treating cancer, comprising administering to a human cancer subject having an IDH mutation a therapeutically effective amount of

(a) a first compound of Formula I:



wherein:

R^1 is $-\text{CH}_2\text{CH}(\text{CH}_3)_2$, $-\text{CH}_2\text{CH}_3$, $-\text{CH}_2\text{CH}_2\text{OCH}_3$, or $-\text{CH}_2$ -cyclopropyl;

R^2 is $-\text{CH}_3$ or $-\text{CH}_2\text{CH}_3$; and

X is N or CH,

or a pharmaceutically acceptable salt thereof; and

(b) a second compound that is a Bcl-2 inhibitor, or a pharmaceutically acceptable salt thereof.

In one embodiment, the IDH mutation is an IDH1 mutation or an IDH2 mutation. In another embodiment, the IDH mutation is an IDH1 mutation. In another embodiment, the IDH1 mutation is an IDH1 R132 mutation. In another embodiment, the IDH1 mutation is R132H, R132C, R132G, R132L, or R132S. In another embodiment, the IDH1 R132 mutation is R132H. In another embodiment, the IDH1 mutation is R132C. In another embodiment, the IDH1 mutation is R132G. In another embodiment, the IDH1 mutation is R132L. In another embodiment, the IDH1 mutation is R132S.

In another embodiment, the IDH mutation is an IDH2 mutation. In another embodiment, the IDH2 mutation is an IDH2 R140 mutation or an IDH2 R172 mutation. In another embodiment, the IDH2 mutation is an R140 mutation. In another embodiment, the R140 mutation is R140Q, R140L, or R140W. In another embodiment, the IDH2 mutation is an R172 mutation. In another embodiment, the R172 mutation is R172K, R172M, R172G, R172S or R172W.

In one embodiment of the method of the invention, in the first compound of Formula I, or a pharmaceutically acceptable salt thereof, X is N,

In another embodiment, in the first compound of Formula I, or a pharmaceutically acceptable salt thereof, X is N, R¹ is -CH₂-cyclopropyl, and R² is -CH₂CH₃. In another embodiment, in the first compound of Formula I, X is N, R¹ is -CH₂-cyclopropyl, and R² is -CH₂CH₃.

In another embodiment, the first compound is:

7-[[[(1S)-1-[4-[(1R)-2-cyclopropyl-1-(4-prop-2-enoyl)piperazin-1-yl)ethyl]phenyl]ethyl]amino]-1-ethyl-4H-pyrimido[4,5-d][1,3]oxazin-2-one;

7-[[[(1S)-1-[4-[(1S)-2-cyclopropyl-1-(4-prop-2-enoyl)piperazin-1-yl)ethyl]phenyl]ethyl]amino]-1-ethyl-4H-pyrimido[4,5-d][1,3]oxazin-2-one; or

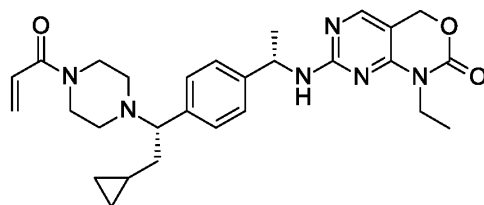
1-Ethyl-7-[[[(1S)-1-[4-[1-(4-prop-2-enoyl)piperazin-1-yl)propyl]phenyl]ethyl]amino]-4H-pyrimido[4,5-d][1,3]oxazin-2-one;

or a pharmaceutically acceptable salt thereof.

In another embodiment, the first compound is 7-[[[(1S)-1-[4-[(1S)-2-cyclopropyl-1-(4-prop-2-enoyl)piperazin-1-yl)ethyl]phenyl]ethyl]amino]-1-ethyl-4H-pyrimido[4,5-d][1,3]oxazin-2-one.

In another embodiment, the first compound is:

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Compound A

or a pharmaceutically acceptable salt thereof. In another embodiment, the first compound is Compound A.

5 In another embodiment, the subject is identified as having an IDH mutation. In another embodiment, the subject is identified as having an IDH mutation in tissue. In another embodiment, the subject is identified as having an IDH1 mutation. In another embodiment, the subject is identified as having an IDH2 mutation.

 In another embodiment, the subject is identified as having an R132 IDH1
10 mutation. In another embodiment, the subject is identified as having an R132 IDH1 mutation in tissue. In another embodiment, the subject is identified as having an IDH2 mutation, *e.g.*, an IDH2 R172, R140 or R172 mutation. In another embodiment, the subject is identified as having an IDH2 R172, R140 or R172 mutation in tissue.

 In another embodiment, the cancer is a hematologic malignancy, and the subject is
15 identified as having an IDH mutation (*e.g.*, an IDH1 R132 mutation or an IDH2 R172, R140 or R172 mutation) in blood, bone marrow, lymph node, lymphatic fluid. In another embodiment, the subject is identified as having an IDH mutation (*e.g.*, an IDH1 R132 mutation or an IDH2 R172, R140 or R172 mutation) in blood cells, bone marrow cells, lymph node cells or lymphatic fluid cells.

20 In another embodiment, the cancer is a solid tumor cancer, and the subject is identified as having an IDH mutation (*e.g.*, an IDH1 R132 mutation or an IDH2 R172, R140 or R172 mutation) in solid tumor tissue. In another embodiment, the subject is identified as having an IDH mutation (*e.g.*, an IDH1 R132 mutation or an IDH2 R172, R140 or R172 mutation) in solid tumor tissue cells.

25 In another embodiment, the first compound, or the pharmaceutically acceptable salt thereof, is administered before the second compound, or a pharmaceutically acceptable salt thereof.

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In another embodiment, the first compound, or the pharmaceutically acceptable salt thereof, is administered after the second compound, or a pharmaceutically acceptable salt thereof.

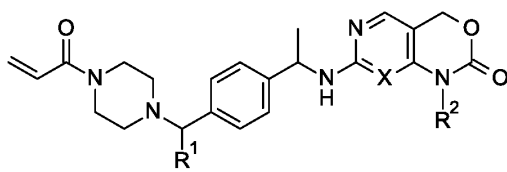
5 In another embodiment, the first compound, or the pharmaceutically acceptable salt thereof, is co-formulated with the second compound, or a pharmaceutically acceptable salt thereof.

In another embodiment of the method of the invention, the cancer is a solid tumor cancer. In another embodiment, the solid tumor cancer is cholangiocarcinoma, head & neck cancer, chondrosarcoma, hepatocellular carcinoma, melanoma, pancreatic cancer, 10 astrocytoma, oligodendroglioma, glioma, glioblastoma, bladder carcinoma, colorectal cancer, lung cancer, or sinonasal undifferentiated carcinoma. In another embodiment, the lung cancer is non-small cell lung cancer. In another embodiment, the solid tumor cancer is cholangiocarcinoma.

In another embodiment, the cancer is a hematologic malignancy. 15 In another embodiment, the hematologic malignancy is acute myeloid leukemia, myelodysplastic syndrome myeloproliferative neoplasm, angioimmunoblastic T-cell lymphoma, T-cell acute lymphoblastic leukemia, polycythemia vera, essential thrombocythemia, primary myelofibrosis, or chronic myelogenous leukemia. In another embodiment, the hematologic malignancy is acute myeloid leukemia.

20 The present invention also provides a compound of Formula I:

I



wherein:

25 R¹ is -CH₂CH(CH₃)₂, -CH₂CH₃, -CH₂CH₂OCH₃, or -CH₂-cyclopropyl;
 R² is -CH₃ or -CH₂CH₃;
 X is N or CH; or a pharmaceutically acceptable salt thereof;

for use in combination with a Bcl-2 inhibitor, or a pharmaceutically acceptable salt thereof, in the treatment of cancer in a human subject having an IDH mutation in blood cells, bone marrow cells, or blood and bone marrow cells.

In one embodiment, the IDH mutation is an IDH1 mutation or an IDH2 mutation.

5 In another embodiment, the IDH mutation is an IDH1 mutation. In another embodiment, the IDH1 mutation is an IDH1 R132 mutation. In another embodiment, the IDH1 mutation is R132H, R132C, R132G, R132L, or R132S. In another embodiment, the IDH1 R132 mutation is R132H. In another embodiment, the IDH1 mutation is R132C. In another embodiment, the IDH1 mutation is R132G. In another embodiment, the IDH1
10 mutation is R132L. In another embodiment, the IDH1 mutation is R132S.

In another embodiment, the IDH mutation is an IDH2 mutation. In another embodiment, the IDH2 mutation is an IDH2 R140 mutation or an IDH2 R172 mutation. In another embodiment, the IDH2 mutation is an R140 mutation. In another embodiment, the R140 mutation is R140Q, R140L, or R140W. In another embodiment, the IDH2
15 mutation is an R172 mutation. In another embodiment, the R172 mutation is R172K, R172M, R172G, R172S or R172W.

In one embodiment, the subject is identified as having an IDH mutation (e.g., an IDH1 R132 mutation or an IDH2 R172, R140 or R172 mutation).

In one embodiment the compound for use is of Formula I, wherein X is N, or a
20 pharmaceutically acceptable salt thereof. In another embodiment, the compound for use is of Formula I, wherein R¹ is -CH₂-cyclopropyl, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound for use is of Formula I, wherein R² is -CH₂CH₃, or a pharmaceutically acceptable salt thereof. In another embodiment, the compound for use is of Formula I, wherein X is N, R¹ is -CH₂-cyclopropyl, and R² is -CH₂CH₃, or a pharmaceutically acceptable salt thereof. In another embodiment, the
25 compound is of Formula I, wherein X is N, R¹ is -CH₂-cyclopropyl, and R² is -CH₂CH₃.

In another embodiment, the compound is:

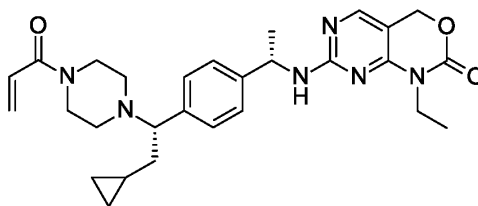
7-[[[(1S)-1-[4-[(1R)-2-Cyclopropyl-1-(4-prop-2-enoyl)piperazin-1-yl)ethyl]phenyl]ethyl]amino]-1-ethyl-4H-pyrimido[4,5-d][1,3]oxazin-2-one;
30 7-[[[(1S)-1-[4-[(1S)-2-cyclopropyl-1-(4-prop-2-enoyl)piperazin-1-yl)ethyl]phenyl]ethyl]amino]-1-ethyl-4H-pyrimido[4,5-d][1,3]oxazin-2-one; or

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1-Ethyl-7-[[[(1S)-1-[4-[1-(4-prop-2-enoylpiperazin-1-yl)propyl]phenyl]ethyl]amino]-4H-pyrimido[4,5-d][1,3]oxazin-2-one;
or a pharmaceutically acceptable salt thereof.

In another embodiment, the compound is 7-[[[(1S)-1-[4-[(1S)-2-cyclopropyl-1-(4-prop-2-enoylpiperazin-1-yl)ethyl]phenyl]ethyl]amino]-1-ethyl-4H-pyrimido[4,5-d][1,3]oxazin-2-one, or a pharmaceutically acceptable salt thereof.

In another embodiment, the compound is:



(Compound A), or a pharmaceutically acceptable salt thereof.

In another embodiment, the compound is Compound A.

Novel methods for use of the combination of a compound of Formula I and a Bcl-2 inhibitor to treat cancer are presented herein. Accordingly, some aspects of the present invention provide for a compound of Formula I for use in simultaneous, separate, or sequential combination with a Bcl-2 inhibitor in the treatment of cancer. Additionally, some aspects of the present invention provide for a compound of Formula I for use in simultaneous, separate, or sequential combination with a Bcl-2 inhibitor in the treatment of a solid tumor cancer. Moreover, some aspects of the present invention provide for a compound of Formula I for use in simultaneous, separate, or sequential combination with a Bcl-2 inhibitor in the treatment of a hematologic malignancy.

The present invention also provides a pharmaceutical composition comprising a compound of Formula I, for use in treating cancer in a human subject having an IDH mutation in blood cells, bone marrow cells, lymph node or lymphatic fluid.

In one embodiment, the IDH mutation is an IDH1 mutation or an IDH2 mutation. In another embodiment, the IDH mutation is an IDH1 mutation. In another embodiment, the IDH1 mutation is an IDH1 R132 mutation. In another embodiment, the IDH1 mutation is R132H, R132C, R132G, R132L, or R132S. In another embodiment, the IDH1 R132 mutation is R132H. In another embodiment, the IDH1 mutation is R132C.

In another embodiment, the IDH1 mutation is R132G. In another embodiment, the IDH1 mutation is R132L. In another embodiment, the IDH1 mutation is R132S.

In another embodiment, the IDH mutation is an IDH2 mutation. In another embodiment, the IDH2 mutation is an IDH2 R140 mutation or an IDH2 R172 mutation.

- 5 In another embodiment, the IDH2 mutation is an R140 mutation. In another embodiment, the R140 mutation is R140Q, R140L, or R140W. In another embodiment, the IDH2 mutation is an R172 mutation. In another embodiment, the R172 mutation is R172K, R172M, R172G, R172S or R172W.

- 10 The present invention also provides the use of a compound of Formula I, in the manufacture of a medicament for the treatment of cancer in a human subject identified as having an IDH mutation (*e.g.*, an IDH1 R132 mutation or an IDH2 R172, R140 or R172 mutation) in blood, bone marrow, lymph node, lymphatic fluid, blood cells, bone marrow cells, lymph node cells or lymphatic fluid cells.

- In one embodiment of the method of the invention, the cancer is frontline cancer.
- 15 In another embodiment, the frontline cancer is a solid tumor cancer. In another embodiment, the frontline cancer is hematologic malignancy. In another embodiment, the frontline hematologic malignancy is frontline AML.

- In another embodiment of the method of the invention, the cancer is relapsed cancer. In another embodiment, the relapsed cancer is a solid tumor cancer. In another
- 20 embodiment, the relapsed cancer is hematologic malignancy. In another embodiment, the relapsed hematologic malignancy is relapsed AML.

- In another embodiment of the method of the invention, the cancer is refractory cancer. In another embodiment, the refractory cancer is a solid tumor cancer. In another
- 25 embodiment, the refractory cancer is hematologic malignancy. In another embodiment, the refractory hematologic malignancy is refractory AML.

- In another embodiment of the method of the invention, the cancer is advanced cancer. In another embodiment, the advanced cancer is an advanced solid tumor cancer. In another embodiment, the advanced cancer is an advanced hematologic malignancy. In
- another embodiment, the advanced hematologic malignancy is advanced AML

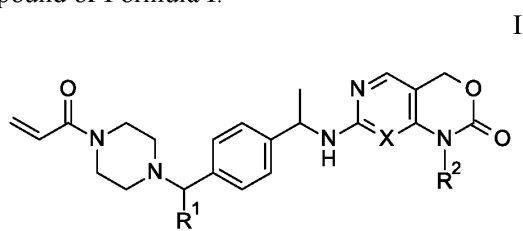
- 30 In another embodiment, the AML is acute promyelocytic leukemia.

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In one embodiment of the the method of the invention, the Bcl-2 inhibitor is venetoclax, obatoclax, navitoclax, or a pharmaceutically acceptable salt of any one of them. In another embodiment, the Bcl-2 inhibitor is venetoclax, or a pharmaceutically acceptable salt thereof. In another embodiment, the Bcl-2 inhibitor is venetoclax.

5 The present invention also provides a method of identifying a human subject for treatment with

(a) a first compound of Formula I:



10 wherein:

R^1 is $-\text{CH}_2\text{CH}(\text{CH}_3)_2$, $-\text{CH}_2\text{CH}_3$, $-\text{CH}_2\text{CH}_2\text{OCH}_3$, or $-\text{CH}_2$ -cyclopropyl;

R^2 is $-\text{CH}_3$ or $-\text{CH}_2\text{CH}_3$; and

X is N or CH,

or a pharmaceutically acceptable salt thereof; and

15 (b) a second compound that is a Bcl-2 inhibitor, or a pharmaceutically acceptable salt thereof,

the method comprising identifying a human subject having a cancer and determining if the human subject has IDH mutation in blood, bone marrow, lymph node, lymphatic fluid, blood cells, bone marrow cells, lymph node cells, lymphatic fluid cells, 20 or solid tissue, wherein the subject has a cancer.

In one embodiment, the IDH mutation is an IDH1 mutation or an IDH2 mutation. In another embodiment, the IDH mutation is an IDH1 mutation. In another embodiment, the IDH1 mutation is an IDH1 R132 mutation. In another embodiment, the IDH1 mutation is R132H, R132C, R132G, R132L, or R132S. In another embodiment, the 25 IDH1 R132 mutation is R132H. In another embodiment, the IDH1 mutation is R132C. In another embodiment, the IDH1 mutation is R132G. In another embodiment, the IDH1 mutation is R132L. In another embodiment, the IDH1 mutation is R132S.

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In another embodiment, the IDH mutation is an IDH2 mutation. In another embodiment, the IDH2 mutation is an IDH2 R140 mutation or an IDH2 R172 mutation. In another embodiment, the IDH2 mutation is an R140 mutation. In another embodiment, the R140 mutation is R140Q, R140L, or R140W. In another embodiment, the IDH2
 5 mutation is an R172 mutation. In another embodiment, the R172 mutation is R172K, R172M, R172G, R172S or R172W.

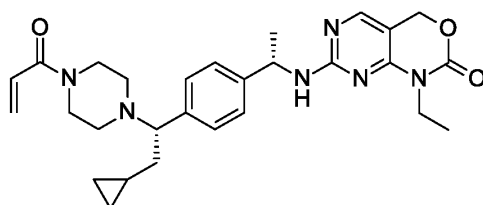
In one embodiment of the method of the invention of identifying a human cancer subject for treatment, in the first compound of Formula I, X is N, or a pharmaceutically acceptable salt thereof. In another embodiment, in the first compound of Formula I, X is
 10 N, R¹ is -CH₂-cyclopropyl, and R² is -CH₂CH₃, or a pharmaceutically acceptable salt thereof. In another embodiment, in the first compound, X is N, R¹ is -CH₂-cyclopropyl, and R² is -CH₂CH₃.

In another embodiment of the method of identifying a human cancer subject for treatment, the first compound is:

15 7-[[[(1S)-1-[4-[(1R)-2-cyclopropyl-1-(4-prop-2-enoyl)piperazin-1-yl)ethyl]phenyl]ethyl]amino]-1-ethyl-4H-pyrimido[4,5-d][1,3]oxazin-2-one;
 7-[[[(1S)-1-[4-[(1S)-2-cyclopropyl-1-(4-prop-2-enoyl)piperazin-1-yl)ethyl]phenyl]ethyl]amino]-1-ethyl-4H-pyrimido[4,5-d][1,3]oxazin-2-one; or
 1-Ethyl-7-[[[(1S)-1-[4-[1-(4-prop-2-enoyl)piperazin-1-yl)propyl]phenyl]ethyl]amino]-4H-pyrimido[4,5-d][1,3]oxazin-2-one;
 20 or a pharmaceutically acceptable salt thereof.

In another embodiment, the first compound is 7-[[[(1S)-1-[4-[(1S)-2-cyclopropyl-1-(4-prop-2-enoyl)piperazin-1-yl)ethyl]phenyl]ethyl]amino]-1-ethyl-4H-pyrimido[4,5-d][1,3]oxazin-2-one.

25 In another embodiment, the first compound is:



(Compound A) or a pharmaceutically acceptable salt thereof. In another embodiment, the first compound is Compound A.

Methods for assaying mutant IDH1 and IDH2 enzyme activity are known to those of ordinary skill in the art, *e.g.*, in WO 2018/111707 A1.

5 As used above, and throughout the description of the invention, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

The term “hematologic tissue” refers to blood, bone marrow, spleen, lymph node, or lymphatic fluid.

The term “solid tumor tissue” refers to tissue that is not hematologic tissue. Non-limiting examples of solid tissue are cholangial tissue, pancreatic tissue, head tissue, neck
10 tissue, hepatic tissue, skin tissue, astrocytoma tissue, oligodendroglial tissue, glial tissue, brain tissue, bladder tissue, colorectal tissue, and lung tissue.

As used herein, a “Bcl-2 inhibitor” is a compound that binds to Bcl-2, and results in one or more of cytotoxicity in cancer cells, downregulation of Bcl-2 expression in
15 cancer cells, mitochondrial dysfunction in cancer cells, and apoptosis in cancer cells. Methods for determining those effects are known to those of ordinary skill in the art, *e.g.*, in Wen M, *et al.*, *Front. Pharmacol.* 2019; 10: 391.

The term “mutant IDH inhibitor” refers to a compound that inhibits the enzyme activity of and/or the production of 2-HG by a mutant IDH enzyme (*e.g.*, a mutant IDH1
20 enzyme or a mutant IDH2 enzyme). Methods for assaying mutant IDH1 and mutant IDH2 enzyme activity are known to those of ordinary skill in the art, *e.g.*, in WO 2018/111707 A1. In the term “mutant IDH inhibitor, the word “mutant” refers to the IDH gene, not the inhibitor.

The term “solid tumor cancer” means that the cancer originated in a tissue that is
25 not blood or bone marrow.

The term “hematologic malignancy” relates to cancer that originated in the blood, bone marrow, lymph node or lymphatic fluid.

The term “frontline cancer” means that the human cancer subject has never been treated for the cancer being treated.

30 The term “refractory cancer” refers to cancer that has been treated, but the human cancer subject did not respond to treatment.

The term “relapsed cancer” means that the human cancer subject responded to treatment for a period of time, but that the cancer has reoccurred.

The term “advanced cancer” refers to cancer that has spread to lymph nodes or to other tissues outside of the cancer’s point of origin. For example, AML is AML that has spread to a tissue outside of the blood or the bone marrow.

The term “cancer subject” means a subject who has been diagnosed with cancer.

The term “solid tumor subject” means a subject who has been diagnosed with a solid tumor cancer. In one embodiment, the solid tumor cancer is cholangiocarcinoma.

The term “hematologic malignancy subject” means a subject who has been diagnosed with a hematologic malignancy. In one embodiment, the hematologic malignancy subject is an AML subject. The term “AML subject” means a subject who has been diagnosed with AML. Methods for diagnosing AML are known to those of ordinary skill in the art, *e.g.*, in Dohner H, *et al.*, *Blood* 2017; 129: 424-447.

The terms “acute myeloid leukemia,” “acute myelogenous leukemia,” and “acute nonlymphocytic leukemia” are synonymous.

“Responsiveness to hematologic malignancy (*e.g.*, AML) treatment” includes improvement in overall survival, partial response to treatment, long-term stable disease, or improvement in long-term survival characterized as complete remission (determined by less than 5% myeloblasts in bone marrow or the absence of circulating blasts), hematologic recovery (as evidenced by a peripheral blood absolute neutrophil count greater than 1,000 cells/ μ L and a platelet count greater than 100,000/ μ L, without the need for red blood cell transfusion, and the absence of extramedullary disease) (Bloomfield CD, *et al.*, *Blood Revs.* 2018; 32: 416-425).

The term “IDH1 R132 mutation” is synonymous with “R132 IDH1 mutation,” and refers to an IDH1 mutation at amino acid residue 132 in a subject’s IDH1 gene., as determined, *e.g.*, in the subject’s nucleic acid (*e.g.*, DNA).

The term “identified as having an IDH mutation” means that nucleic acid (*e.g.*, DNA) from a human subject’s tissue or cells has been analyzed to determine if the human subject has an IDH mutation (*e.g.*, an IDH1 R132 mutation, an IDH2 R140 mutation or an IDH2 R172 mutation). In one embodiment, the human subject’s blood, blood cells, bone marrow, bone marrow cells, lymph node, lymph node cells, lymphatic fluid or

lymphatic fluid cells has been analyzed for an IDH mutation. In another embodiment, the human subject's solid tissue has been analyzed for an IDH mutation. In another embodiment, the human subject's solid tissue has been analyzed for an IDH mutation.

In the methods of the present invention, the party who identifies the human
5 subject as having an IDH mutation (*e.g.*, an IDH1 R132 mutation, an IDH2 R140 mutation or an IDH2 R172 mutation) can be different than the party that administers the first and second compounds. In one embodiment, the party who identifies the human subject as having an IDH mutation (*e.g.*, an IDH1 R132 mutation, an IDH2 R140 mutation or an IDH2 R172 mutation) is different than the party that administers the first
10 and second compounds.

Analytical methods identifying genetic mutations are known to those of ordinary skill in the art (Clark, O., *et al.*, *Clin. Cancer. Res.* 2016; 22: 1837-42), including, but not limited to, karyotyping (Guller JL, *et al.*, *J. Mol. Diagn.* 2010; 12: 3-16), fluorescence *in situ* hybridization (Yeung DT, *et al.*, *Pathology* 2011; 43: 566-579), Sanger sequencing
15 (Lutha, R *et al.*, *Haematologica* 2014; 99: 465-473), metabolic profiling (Miyata S, *et al.*, *Scientific Reports* 2019; 9: 9787), polymerase chain reaction (Ziai, JM and AJ Siddon, *Am. J. Clin. Pathol.* 2015; 144: 539-554), next-generation sequencing (*e.g.*, whole transcriptome sequencing) (Lutha, R *et al.*, *Haematologica* 2014; 99: 465-473; and Wang H-Y, *et al.*, *J. Exp. Clin. Cancer Res.* 2016; 35: 86).

20 The term "in combination with" means that a compound of Formula I is used, or is for use, in simultaneous, separate, or sequential combination with a Bcl-2 inhibitor in the treatment of cancer.

The terms "treatment," "treat," "treating," and the like, are meant to include slowing, stopping, or reversing the progression of cancer. These terms also include
25 alleviating, ameliorating, attenuating, eliminating, or reducing one or more symptoms of a disorder or condition, even if the cancer is not actually eliminated and even if progression of the cancer is not itself slowed, stopped or reversed.

"Therapeutically effective amount" means the amount of a compound, or pharmaceutically acceptable salt thereof, administered to the subject that will elicit the
30 biological or medical response of or desired therapeutic effect on a subject. A therapeutically effective amount can be readily determined by the attending clinician, as

one skilled in the art, by the use of known techniques and by observing results obtained under analogous circumstances. In determining the effective amount for a subject, a number of factors are considered by the attending clinician, including, but not limited to: size, age, and general health; the specific disease or disorder involved; the degree of or
5 involvement or the severity of the disease or disorder; the response of the individual subject; the particular compound administered; the mode of administration; the bioavailability characteristics of the preparation administered; the dose regimen selected; the use of concomitant medication; and other relevant circumstances.

The compounds administered in the method of the invention can optionally be
10 formulated as pharmaceutical compositions administered by any route which makes the compounds bioavailable. In an embodiment, such compositions are formulated for oral administration. Such pharmaceutical compositions and processes for preparing the same are well known in the art. (See, e.g., Remington: The Science and Practice of Pharmacy (D.B. Troy, Editor, 21st Edition, Lippincott, Williams & Wilkins, 2006).

15 A "pharmaceutically acceptable carrier, diluent, or excipient" is a medium generally accepted in the art for the delivery of biologically active agents to mammals, e.g., humans.

It will be understood by one of ordinary skill in the art that compounds administered in the method of the invention are capable of forming salts. The compounds
20 react with any of a number of inorganic and organic acids to form pharmaceutically acceptable acid addition salts. Such pharmaceutically acceptable acid addition salts and common methodology for preparing them are well known in the art. See, e.g., P. Stahl, *et al.*, HANDBOOK OF PHARMACEUTICAL SALTS: PROPERTIES, SELECTION AND USE, (VCHA/Wiley-VCH, 2008).

25 "Pharmaceutically acceptable salts" or "a pharmaceutically acceptable salt" refers to the relatively non-toxic, inorganic and organic salt or salts of the compounds of the present invention (S.M. Berge, *et al.*, "Pharmaceutical Salts", *Journal of Pharmaceutical Sciences*, Vol 66, No. 1, January 1977).

Materials and Methods

2-HG Inhibition Assay

- Cell line construction.** MOLM14 wild-type human leukemia cells and a MOLM14_R132 construct cell line are generated by lentiviral transfection of MOLM14 cells using IDH1 WT (doxycycline inducible) pSLIK-IDH1-FLAG (Addgene Plasmid #66802) and IDH1 R132H (doxycycline inducible) pSLIK-IDH1-R132H-FLAG (Addgene Plasmid #66803), and is kindly provided by Dr. Jean-Emmanuel Sarry (Centre de Recherches en Cancérologie de Toulouse, UMR1037, Inserm, Université de Toulouse 3 Paul Sabatier, Toulouse, France; bioRxiv 749580; doi.org/10.1101/749580).
- MOLM14-WT and MOLM14-R132 mutant cells are seeded in 6 well plates (1×10^6 / well) in 5 ml of complete RPMI medium containing 10% FBS (Sigma) and 1x Pen/Strep (Sigma). After induction with 2 μ g/ml doxycycline, the cells are incubated in 37 degree for 4 days.
- Cells are seeded in 12 wells plates (100,000 cells /well) in 1mL, in triplicate. Cells are treated with 2 μ g/ml doxycycline to induce IDH1 expression, then treated with DMSO, 0.1 or 1 μ M Compound A, and incubated at 37 degrees C for 4 days.
- 65 μ L of media are collected and centrifuged, and 60 μ L of the supernatant media are collected and frozen at -80 degrees C.
- LCMS 2-HG metabolite analysis.** The effects of IDH1 inhibition on the concentrations of total 2-HG and (α -KG) are determined by liquid chromatography-mass spectrometry (LC-MS) analysis of cell culture supernatant. Calibration curves are prepared by spiking 2-HG and α -KG into cell culture media. The method utilizes derivatization with O-benzylhydroxylamine prior to analysis by LC-MS. Ten microliters of each standard or sample is placed into a deep-well 96-well plate and combined with 100 μ L of internal standard solution containing 10 μ M d5-2-hydroxyglutarate and 10 μ M d6- α -KG. 50 μ L of 1 M O-benzylhydroxylamine in pyridine buffer (8.6% pyridine, pH 5) and 50 μ L of 1 M N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) in pyridine buffer is added to each sample. The derivatization reaction proceeds at room temperature for one hour. Using a Beckman Biomek FX liquid handler 300 μ L of ethyl acetate is added to each sample. Plates are sealed and vortexed for 5 minutes, and then centrifuged for 5

- minutes at 4000 rpm on an Eppendorf 5810R centrifuge. 220 μ L of the upper layer is transferred to a new 96-well plate. Samples are dried under heated nitrogen at 50°C and reconstituted with 100 μ L of methanol/water (1:1). One microliter of derivatized sample is injected onto an LC-MS system consisting of a Shimadzu Prominence 20A HPLC
- 5 system and a Thermo Quantum Ultra™ triple quadrupole mass spectrometer. Analytes are separated on a Water XBridge™ C18 column (2.1 x 50 mm, 3.5 μ m) with a flow rate of 0.6 mL/minute. Mobile phase A is 0.1% formic acid in water and mobile phase B is methanol. The gradient profile is: 0 minutes, 5% B; 2 minutes, 100% B; 4.00 minutes, 100% B; 4.1 minutes, 5% B; 5.50 minutes, stop. The mass spectrometer utilizes a HESI-
- 10 II probe operated in positive ion selected reaction monitoring mode. Calibration curves are constructed by plotting analyte concentrations vs. analyte/internal standard peak area ratios and performing a quadratic fit of the data using a 1/concentration weighting with Xcalibur™ software. Analyte concentrations for the unknowns are back-calculated from the calibration curves.
- 15 In experiments performed essentially as described above, the results in Table 1 are obtained.

Table 1. 2-HG (μM)		
DMSO	0.1 μM Compound A	1 μM 20 Compound A
44.308	3.369	3.578
53.071	3.044	3.199
54.163	3.488	3.378

- 25 The results in Table 1 demonstrate that in the MOLM14_R132 cell line construct, Compound A inhibits 2-HG production by mutant IDH1.

Cell Viability Assay

- 30 MOLM14 wild-type and MOLM14_R132 cells are treated with 2 μ g/ml doxycycline to induce expression of IDH1 WT and IDH1-R132H and then incubated at 37 degrees C for 4 days. Cells are seeded in 4 (12 wells) plates at 10,000 cells/well in 1mL growth media containing 2ug/mL doxycycline.

Cells are treated with: DMSO (in triplicate), or with Compound A at 0, 125, 250, 500, 1000 nM (each in triplicate) as described in the following: After the initial induction described above, 0.5 mL media with 1x of doxycycline plus 1x Compound A or DMSO is added and incubated for 3 days. After the 3 days, another 0.5mL aliquot of fresh media with 1X doxycycline plus 1X compound A or DMSO is added and incubated for 3 further days. Venetoclax (0, 25, 50, 100, 200 nM) is then added as a single agent or in combination with 1X Compound A. Two days later, cells are collected in flow cytometry filtered tubes, and processed for flow cytometry. Cells are washed with Annexin V Binding Buffer (ABB, 2 mL) [1 M Hepes buffer (10 mL), 5 M NaCl (28 mL), 1 M CaCl₂ (5 mL), H₂O (957 mL)], and then centrifuged at 1500 rpm for 5 min. Cells are stained with ABB (50 µL), with APC Annexin V Antibody (1 µL, Biolegend cat#640941), and kept in the dark for 20 minutes. Cells are washed with ABB (2 mL) and centrifuged at 1500 rpm for 5 min. DAPI (Invitrogen cat#D3571) and counting beads (Invitrogen cat#C36950) are added in Annexin V binding buffer [DAPI (5 µL, 2µg/mL) + counting beads (5 µL (520,000 beads per 50 µL)) + ABB (150 µL)] in a total volume 160 µL/tube, then analyzed with a Gallios flow cytometer (Beckman Coulter). Cells collection is stopped when counting beads reach 250 beads per sample. Kalusa software is used for analysis. The percentage of gated Annexin V-negative/ DAPI-negative cells constitutes % viable cells, while the percentage of Annexin V-positive cells constitutes % apoptosis.

In experiments performed essentially as described above, the results in Tables 2A-2C and 3 are obtained.

Table 2A. Cell viability after treatment with Compound A			
Compound A (nM)	% Cell Viability		
0	96.63	96.2	96.25
125	93.72	95.14	94.27
250	93.34	94.27	94.41
500	93.66	93.92	94.15
1000	92.03	93.98	91.68

Table 2B. % Cell viability after treatment with Venetoclax			
venetoclax (nM)	% Cell Viability		
0	96.63	96.2	96.25
25	76.09	81.78	77.84
50	76.91	80.63	78.78
100	70.42	76.91	73.78
200	55.2	59.27	56.31

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Table 2C. % Cell viability after treatment with Combination of Compound A and Venetoclax			
venetoclax (nM) and Compound A (nM), respectively	% Cell Viability		
0, 0	96.63	96.2	96.25
25, 125	69.03	76.03	74.44
50, 250	71.16	77.27	73.85
100, 500	60.22	66.97	63.16
200, 1000	27.26	34.96	27.25

The results in Tables 2A-2C demonstrate that the combination of venetoclax and Compound A results in further reduction in leukemic cell viability, relative to the level of leukemic cell viability of cells treated with either compound alone.

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Table 3. %Annexin V+ as a measure of apoptosis									
Venetoclax (nM) and Compound A (nM), respectively	Compound A			Venetoclax			Compound A + Venetoclax		
0, 0	2.89	2.94	2.88	2.89	2.94	2.88	2.89	2.94	2.88
25, 125	5.56	4.3	5.12	19.1	15.93	19.16	26.41	19.75	22.89
50, 250	6.18	4.95	4.92	18.22	16.34	17.15	24.65	17.31	19.45
100, 500	5.82	5.23	5.19	24.62	18.98	22.98	30.57	28.46	26.32
200, 1000	7.2	5.51	7.52	41.67	35.73	41.22	69.28	55.11	67.71

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15 The results in Table 3 demonstrate that the combination of venetoclax and Compound A results in an increased level of apoptosis in leukemic cells, relative to the level of apoptosis of cells treated with either compound alone.

AML-PDX model

20 Male NSG mice (6-9 weeks of age, The Jackson Laboratory) are irradiated with 250 cGy and on the next day, injected intravenously with AML-PDX (1×10^6 cells/100 μ L). Peripheral blood is collected via the retro-orbital route and processed to measure hCD45+ cells by flow cytometry to confirm the establishment of leukemia.

When leukemia engraftment reaches more than 1%, mice are randomly grouped
25 for treatment with vehicle, Compound A (10 mg/kg, daily oral gavage), venetoclax (50

mg/kg, oral gavage for 2 weeks on and 1 week off (2 cycles)) or the combination of venetoclax (50 mg/kg, oral gavage for 2 weeks on and 1 week off (2 cycles)) and Compound A (10 mg/kg, daily oral gavage). Additional dosing may be employed beyond the 2 cycles stated above based on the assessment of the level of residual disease

5 remaining following 6 weeks of treatment.

Peripheral blood is collected once every two weeks via the retro-orbital route and processed to measure leukemia burden (%hCD33+/hCD45+ cells) by flow cytometry, and to measure differentiation (%hCD14+ cells percentage out of total hCD45+ cells).

Sixty-five μ L stain/wash buffer (5% heat inactivated (HI)-FBS in DPBS) is added per
 10 tube, mixed up and down 3 to 4 times by pipette, and 100 μ L of the blood suspension is transferred to 5 mL polypropylene tubes. The stains are added according to the manufacturer's instructions (anti-human CD33-APC (BD Biosciences cat#551378), anti-human CD14-PE-Cy7 (BD Biosciences cat#557742), anti-human CD45-APC-Cy7 (BD Biosciences cat#557833). The samples are incubated for 30 minutes at room temperature,
 15 protected from light. After the incubation, 1.5 mL 1X BD Lyse/Fix buffer (37°C, (BD#558049)) is added to each sample and incubated for 12 minutes at room temperature. The tubes are then centrifuged at 1500 RPMI for 5 minutes. BD Lyse/Fix solution is aspirated and the cell pellet washed with stain/wash buffer 2 times. Fixed cells are then resuspended in 200 μ L stain/wash buffer then transferred to filtered flow tubes.
 20 Samples are analyzed on a Gallios Flow cytometer (Beckman, Texas) using standard flow cytometry principles and technique. Population gating and data analysis of percent population are performed in the Flow Jo software.

In experiments performed essentially as described above, the results in Tables 4 and 5 are obtained.

Table 4. AML cell differentiation (%hCD14+ of hCD45+)				
Animal	Vehicle	Compound A	Venetoclax	Compound A + Venetoclax
1	20.6	63.6	37.7	55.2
2	25	64.1	26	68.5
3	34.6	51.7	40.7	56.7
4	20.7	58.9	32.6	54.1
5	19.4	44.1	ND	71.1
6	ND	58.3	ND	ND

25 ND: not determined (Data collected following 6 weeks of treatment)

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Table 5. Leukemic burden (%hCD33+hCD45+ of total white cells)				
Animal	Vehicle	Compound A	Venetoclax	Compound A + Venetoclax
1	74	51.5	10.1	6.4
2	86.5	65.6	63	15.2
3	92.6	85.1	14.1	5.25
4	93.3	81.9	54.9	4.73
5	92.6	87.8	ND	5.91
6	ND	88.5	ND	ND

ND: not determined (Data collected following 6 weeks of treatment)

- The results in Tables 4 and 5 demonstrate that even though the combination of Venetoclax and Compound A does not result in an increased level of AML cell
- 5 differentiation, the combination does result in a lower leukemia burden, relative to leukemia burden obtained using either compound alone, and reducing leukemia burden is a relevant clinical endpoint.