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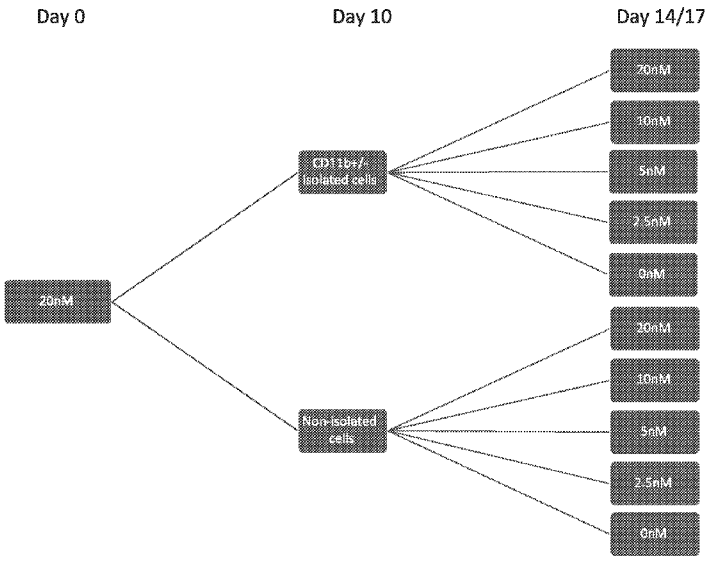
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(54) Title: DIFFERENTIATION AND MAINTENANCE DOSING REGIMEN FOR FHD 286 (BRG1/BRM INHIBITOR)

FIG. 1



(57) Abstract: The present disclosure relates to FHD 286 dosing regimens for the treatment of AML and related hematological cancers. The therapies of the invention can reduce side effects, improve outcomes, and/or reduce the dosing burden on the patient.

[Continued on next page]

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DIFFERENTIATION AND MAINTENANCE DOSING REGIMEN FOR FHD 286 (BRG1/BRM INHIBITOR)

BACKGROUND OF THE INVENTION

The present disclosure relates to FHD 286 dosing regimens for the treatment of cancer, including AML and related hematological cancers and solid tumors. The therapies of the invention can reduce side effects, improve outcomes, and/or reduce the dosing burden on the patient.

SUMMARY

The present disclosure features a method of administering to a subject (i) a differentiation dose regimen of FHD 286, or a pharmaceutically acceptable salt thereof, and, following step (i), administering to the subject (ii) maintenance dose regimen of FHD 286, or a pharmaceutically acceptable salt thereof, wherein the differentiation dose regimen and the maintenance dose regimen together are effective to treat the cancer, and wherein the average daily dose during the differentiation dose regimen is at least 1.25-fold higher than the average daily dose during the maintenance dose regimen.

In some embodiments, the differentiation dose regimen (a) increases the appearance of a marker of differentiation in one or more dedifferentiated cells of the cancer, (b) decreases the appearance of a marker of dedifferentiation in one or more dedifferentiated cells of the cancer, (c) induces cell arrest in one or more dedifferentiated cells of the cancer, or (d) induces apoptosis in in one or more dedifferentiated cells of the cancer.

In some embodiments, following the administration of the differentiation dose regimen, the maintenance dose regimen inhibits the formation of dedifferentiated cells of the cancer.

In some embodiments, the differentiation dose regimen is sufficient to produce a $C_{average}$ plasma concentration level of at least 250 ng/mL in the subject, and the maintenance dose regimen is sufficient to produce a $C_{average}$ plasma concentration level of between about 80 ng/mL and about 225 ng/mL in the subject.

The differentiation dose regimen can include administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject once or more every ten days to fourteen days, once or more weekly, or administration daily. In certain embodiments, (i) the differentiation dose of FHD 286 is administered to the subject at least once daily for one to five days, at least once daily for one to seven days, at least once daily for one to ten days, at least once daily for one to fourteen days, at least once daily for one to twenty-eight days, or the differentiation dose of FHD 286 is administered to the subject daily for one day.

In certain embodiments, the maintenance dose of FHD 286 is administered to the subject at least once daily for one to twenty-eight days. In certain embodiments, the maintenance dose of FHD 286 is administered to the subject at least once daily. In particular embodiments, the differentiation dose of FHD 286 is administered, followed by six to eight days without administration of FHD 286.

In particular embodiments, the differentiation dose of FHD 286 is administered, followed by seven days without administration of FHD 286. In particular embodiments, the differentiation dose of FHD 286 is administered, followed by one day without administration of FHD 286. In some embodiments, the differentiation dose and the maintenance dose are administered within 28 days of each other, within 14

days of each other, within 9 days of each other, within 5 days of each other, within 2 days of each other, or within 24 hours of each other.

The average daily dose during the differentiation regimen is at least 1.25-fold (e.g., at least 1.3-fold, 1.2-fold, or 2.0-fold) higher than the average daily dose during the maintenance regimen.

In particular embodiments, the differentiation dose regimen comprises administering daily to the subject greater than 7.5 mg to 22.5 mg of FHD 286, or a pharmaceutically acceptable salt thereof, and the maintenance dose regimen comprises administering daily to the subject from 1.5 mg to 7.5 mg of FHD 286, or a pharmaceutically acceptable salt thereof.

In particular embodiments, the differentiation dose regimen is sufficient to produce a $C_{average}$ plasma concentration level of at least 250 ng/mL in the subject, and the maintenance dose regimen is sufficient to produce a $C_{average}$ plasma concentration level of between about 80 ng/mL and about 225 ng/mL in the subject.

In particular embodiments, the method includes administering a differentiation dose regimen including an average daily dose between about greater than 7.5 mg and about 22.5 mg per day of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject (e.g., between about 8 mg and about 10 mg per day, between about 8 mg and about 15 mg per day, between about 10.0 mg and about 12.5 mg per day, between about 10.0 mg and about 15.0 mg per day, between about 10.0 mg and about 17.5 mg per day, between about 15 mg and about 22.5 mg per day, or between about 10 mg and about 20 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof). For example, the method can include a differentiation dose regimen that includes administering to the subject about 10 mg per day, about 12.5 mg per day, about 15 mg per day, about 17.5 mg per day, about 20 mg per day, or about 22.5 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof. In particular embodiments, the differentiation dose regimen can include administration to the subject daily, once in two days, once in three days, once in four days, once in five days, once in six days, once in a week, once in ten days, once in fourteen days or once in twenty-eight days. In particular embodiments, the differentiation dose regimen can include administration to the subject daily, about 10 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof. In particular embodiments, the differentiation dose regimen can include administration to the subject daily, about 12.5 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof. In particular embodiments, the differentiation dose regimen can include administration to the subject daily, about 15 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof. In particular embodiments, the differentiation dose regimen can include administration to the subject daily, about 20 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof. In particular embodiments, the differentiation dose regimen can include administration to the subject daily, an average daily dose of about 10 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof. In particular embodiments, the differentiation dose regimen can include administration to the subject daily, an average daily dose of about 12.5 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof. In particular embodiments, the differentiation dose regimen can include administration to the subject daily, an average daily dose of about 15 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof. In particular embodiments, the differentiation dose regimen can include administration to the subject daily, an average daily dose of about 20 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof.

In particular embodiments, the average daily dose of from greater than 7.5 mg to 22.5 mg of the differentiation dose of FHD 286 or a pharmaceutically acceptable salt thereof, is orally administered to the subject in a unit dosage form (e.g., an average daily dose of from 8 to 10 mg, 8 to 15 mg, 10 to 12.5 mg, 10 to 15 mg, 10 to 17.5 mg, 10 to 20 mg, 10 to 22.5 mg 10 to 25 mg, 12.5 to 15 mg, 12.5 to 17.5 mg, 12.5 to 20 mg, 12.5 to 22.5 mg, 12.5 to 25 mg, 15 to 17.5 mg, 15 to 20 mg, 15 to 22.5 mg, 15 to 25 mg, 17.5 to 20 mg, 17.5 to 22.5 mg, 17.5 to 25 mg of FHD 286 or a pharmaceutically acceptable salt thereof; or an average daily dose of 10 ± 1mg, 12.5 ± 1 mg, 15 ± 1mg, 17.5 ± 1mg, 20 ± 1mg, 22.5 ± 1mg. 25 ± 1mg of FHD 286 or a pharmaceutically acceptable salt thereof). FHD 286 or a pharmaceutically acceptable salt thereof can be administered to the subject daily, 1 to 6 times per week (e.g., once a week, 2 times a week, 3 times a week, 4 times a week, 5 times a week, or 6 times a week), once in two weeks (e.g., one time in the first week and no times in the second week), twice in two weeks (e.g. one time per week for 2 weeks, or 2 times in the first week and no times in the second week), once in three weeks (e.g. one time in the first week and no times in the second or third week), two times in three weeks (e.g. one time in the first week, one time in the second week, and no times in the third week; two times in the first week and no times in the second or third week; or one time in the first week, no times in the second week and one time in the third week) or three times in three weeks (e.g. one time per week for 3 weeks; or 2 times in the first week, one time in the second week and no times in the third week; or three times in the first week and no times in the second or third week; or 2 times in the first week, no time in the second week and one time in the third week), once in four weeks (e.g., one time in the first week and no times in the second, third or fourth week), or four times in four weeks (e.g. one time per week for 4 weeks; or 2 times in the first week, one time in the second week and no times in the third or fourth week; or four times in the first week and no times in the second, third or fourth week; or 2 times in the first week, no time in the second week and one time in the third week and one time in the fourth week; 2 times in the first week, no time in the second or third week and two times in the fourth week; 2 times in the first week, no time in the second week, two times in the third week and no times in the fourth week), once every 28 days (e.g., one time on day 1 and no more times on day 2-28), or 2 to 27 times in 28 days (e.g. one time on day 1 and one or more times of any of the following days: day 2, day 3, day 4, day 5, day 6, day 7, day 8 day 9, day 10, day 11, day 12, day 13, day 14, day 15, day 16, day 17, day 18, day 19, day 20, day 21, day 22, day 23, day 24, day 25, day 26, day 27, or day 28). In preferred embodiments, the differentiation dose regimen comprises administering FHD 286, or a pharmaceutically acceptable salt thereof, in an amount sufficient to produce an average FHD 286 circulating plasma concentration of at least 250 ng/mL to about 1000 ng/mL (e.g., a circulating plasma concentration of about 250 ng/mL, about 300 ng/mL, about 325 ng/mL, about 350 ng/mL, about 375 ng/mL, about 400 ng/mL, about 450 ng/mL, about 500 ng/mL, about 550 ng/mL, about 600 ng/mL, about 750 ng/mL, about 1000 ng/mL).

In particular embodiments, the method includes administering a maintenance dose regimen including an average daily dose between about 1.5 mg and about 7.5 mg per day of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject (e.g., between about 1 mg and about 7.5 mg per day, between about 1.5 mg and about 5 mg per day, between about 1.5 mg and about 5 mg per day, between about 2.5 mg and 5 mg per day, between about 2.5 mg and about 7.5 mg per day, between about 5 mg and about 7.5 mg per day). For example, the maintenance dose regimen can include administering to the subject about 1.5 mg per day, 2.5 mg per day, 5.0 mg per day, about 7.5 mg per day

of the FHD 286, or a pharmaceutically acceptable salt thereof. In particular embodiments, the maintenance dose regimen can include administration to the subject daily, about 5 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof. In particular embodiments, the maintenance dose regimen can include administration to the subject daily, about 7.5 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof. In particular embodiments, the maintenance dose regimen can include administration to the subject daily, an average daily dose of about 5 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof. In particular embodiments, the maintenance dose regimen can include administration to the subject daily, an average daily dose of about 7.5 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof.

In another aspect, following the administration of the differentiation dose regimen, the maintenance dose regimen inhibits the proliferation of dedifferentiated cells of the cancer.

The maintenance dose regimen can include administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of at least one month, at least 3 months, at least six months, or at least 12 months.

The maintenance dose regimen can include administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject once or more every ten to fourteen days, once or more weekly, or at least once daily. In particular embodiments, the maintenance dose regimen comprises a method wherein FHD 286 or a pharmaceutically acceptable salt thereof can be administered to the subject daily, once in two days, once in three days, once in four days, once in five days, once in six days, once in a week, once in ten days, once in fourteen days or once in twenty-eight days.

In particular embodiments, the average daily dose of from 1.5 mg to 7.5 mg of the maintenance dose of FHD 286 or a pharmaceutically acceptable salt thereof, is orally administered to the subject in a unit dosage form (e.g., an average daily dose of from 1.5 to 7.5 mg, 1.0 to 5 mg, 1.0 to 2.5 mg, of FHD 286 or a pharmaceutically acceptable salt thereof; or an average daily dose of 1.5 ± 1mg, 2.5 ± 1 mg, 5 ± 1mg, 7.5 ± 1mg, of FHD 286 or a pharmaceutically acceptable salt thereof). FHD 286 or a pharmaceutically acceptable salt thereof can be administered to the subject daily, 1 to 7 times per week (e.g., once a week, 2 times a week, 3 times a week, 4 times a week, 5 times a week, 6 times a week, 7 times a week), once in two weeks (e.g., one time in the first week and no times in the second week), twice in two weeks (e.g. one time per week for 2 weeks, or 2 times in the first week and no times in the second week), once in three weeks (e.g. one time in the first week and no times in the second or third week), two times in three weeks (e.g. one time in the first week, one time in the second week, and no times in the third week; two times in the first week and no times in the second or third week; or one time in the first week, no times in the second week and one time in the third week) or three times in three weeks (e.g. one time per week for 3 weeks; or 2 times in the first week, one time in the second week and no times in the third week; or three times in the first week and no times in the second or third week; or 2 times in the first week, no time in the second week and one time in the third week), once in four weeks (e.g., one time in the first week and no times in the second, third or fourth week), or four times in four weeks (e.g. one time per week for 4 weeks; or 2 times in the first week, one time in the second week and no times in the third or fourth week; or four times in the first week and no times in the second, third or fourth week; or 2 times in the first week, no time in the second week and one time in the third week and one time in the fourth week; 2 times in the first week, no time in the second or third week and two times in

the fourth week; 2 times in the first week, no time in the second week, two times in the third week and no times in the fourth week), once every 28 days (e.g., one time on day 1 and no more times on day 2-28), or 2 to 27 times in 28 days (e.g. one time on day 1 and one or more times of any of the following days: day 2, day 3, day 4, day 5, day 6, day 7, day 8 day 9, day 10, day 11, day 12, day 13, day 14, day 15, day 16, day 17, day 18, day 19, day 20, day 21, day 22, day 23, day 24, day 25, day 26, day 27, or day 28).

In preferred embodiments, the maintenance dose regimen comprises administering FHD 286, or a pharmaceutically acceptable salt thereof, in an amount sufficient to produce an average FHD 286 circulating plasma concentration of from 10 ng/mL to 225 ng/mL (e.g., a circulating plasma concentration of about 10 ng/mL, about 50 ng/mL, about 75 ng/mL, about 100 ng/mL, about 125 ng/mL, about 150 ng/mL, about 175 ng/mL, about 200 ng/mL).

In preferred embodiments, the ratio of the average daily dose of FHD 286, or a pharmaceutically acceptable salt thereof, administered during the differentiation dose regimen to the average daily dose of FHD 286, or a pharmaceutically acceptable salt thereof, administered during the maintenance dose regimen is from 10:1 to 1.25:1 (e.g., 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1 or 1.25:1).

In some embodiments, the invention provides a method of treating hematological cancer in a subject, wherein the differentiation dose of FHD 286 (high dose) is administered at a dose between about 7.5 mg and 22.5 mg per day by oral administration for one to twenty-eight days. The differentiation dosing cycle is followed by the maintenance dosing cycle dosing cycle. In certain embodiments, the differentiation-maintenance cycle is performed once, twice, or three times.

In a related aspect, the invention features a method of treating hematologic cancer in a subject in need thereof, the method including (a) providing a subject previously treated with an anticancer agent to induce cell arrest and/or apoptosis in leukemic stem cells in the subject, and (b) administering to the subject a maintenance dose regimen of FHD 286, or a pharmaceutically acceptable salt thereof, sufficient to reduce the number of blast cells in the subject. In particular embodiments, the method includes administering a maintenance dose regimen including an average daily dose between about 1.5 mg and about 7.5 mg per day of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject (e.g., between about 1.5 mg and about 7.5 mg per day, between about 1.5 mg and about 5 mg per day, or between about 1.5 mg and about 2.5 mg per day). For example, the maintenance dose regimen can include administering to the subject about 1.5 mg per day, 2.5 mg per day, 5.0 mg per day, about 7.5 mg per day of the FHD 286, or a pharmaceutically acceptable salt thereof. The maintenance dose regimen can include administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of at least one month, at least 3 months, at least six months, or at least 12 months. The maintenance dosing regimen utilized can be any described herein.

In some embodiments, the invention provides a method of treating hematological cancer in a subject, wherein the maintenance dose of FHD 286 (low dose) is administered at a dose between about 1.5 mg and 7.5 mg per day by oral administration for one to twenty-eight days. The maintenance dosing cycle is optionally followed by a subsequent differentiation dosing cycle. In certain embodiments, the differentiation-maintenance dosing cycle is performed once, twice, or three times. The maintenance dosing cycle is optionally followed by a subsequent maintenance dosing cycle. In certain embodiments, the maintenance-maintenance dosing cycle is performed once, twice, or three times.

In some embodiments, the method includes at least 21 days, 28 days, 2 months, 3 months, 4 months, 6 months, 1 year, 2 years, 5 years, or 10 years of treatment.

In some embodiments of any of the above methods, the FHD 286, or a pharmaceutically acceptable salt thereof, is administered orally.

In some embodiments, the invention provides a method of treating hematological cancer in a subject, wherein the risk of developing differentiation syndrome is reduced in the subject relative to treatment with a high dose of FHD 286, or a pharmaceutically acceptable salt thereof, or relative to treatment with a low dose of FHD 286, or a pharmaceutically acceptable salt thereof, alone.

In some embodiments of any of the above methods, the invention provides a method of depleting or promoting differentiation of blast cells of the leukemia in a subject in need thereof, the method comprising administering to the subject (i) a differentiation dose of FHD 286, or a pharmaceutically acceptable salt thereof, and (ii) a maintenance dose of FHD 286, or a pharmaceutically acceptable salt thereof, each in an amount that together is effective in treating depleting or promoting differentiation of blast cells of the leukemia in the subject.

In some embodiments of any of the above methods, the hematological cancer is characterized by differentiation arrest. In some embodiments of any of the above methods, the subject has been treated or is being treated for a hematological cancer is characterized by differentiation arrest.

In some embodiments of any of the above methods, the hematologic cancer is multiple myeloma, large cell lymphoma, acute T-cell leukemia, acute myeloid leukemia, myelodysplastic syndrome, immunoglobulin A lambda myeloma, diffuse mixed histiocytic and lymphocytic lymphoma, B-cell lymphoma, acute lymphoblastic leukemia, diffuse large cell lymphoma, or non-Hodgkin's lymphoma.

In some embodiments of any of the above methods, the hematologic cancer is acute myeloid leukemia, acute myelocytic leukemia, acute myelogenous leukemia, acute granulocytic leukemia, or acute non-lymphocytic leukemia.

In some embodiments of any of the aspects disclosed herein, the hematologic cancer is multiple myeloma, large cell lymphoma, acute T-cell leukemia, acute myeloid leukemia, myelodysplastic syndrome, immunoglobulin A lambda myeloma, diffuse mixed histiocytic and lymphocytic lymphoma, B-cell lymphoma, acute lymphoblastic leukemia, acute promyelocytic leukemia, diffuse large B cell lymphoma, or non-Hodgkin's lymphoma. In some embodiments of any of the aspects disclosed herein, the cancer is diffuse large B cell lymphoma (DLBCL). In some embodiments of any of the aspects disclosed herein, the cancer is acute myeloid leukemia (AML). In some embodiments of any of the aspects disclosed herein, the AML is acute promyelocytic leukemia, arises from a pre-existing myelodysplastic syndrome or myeloproliferative disease, treatment-related AML, AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes, therapy-related myeloid neoplasms, myeloid sarcoma, myeloid proliferations related to Down syndrome, blastic plasmacytoid dendritic cell neoplasm, AML minimally differentiated, AML without maturation, AML with granulocytic maturation, myelomonocytic together with bone marrow eosinophilia, acute monoblastic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, or acute basophilic leukemia. In some embodiments of any of the aspects disclosed herein, the AML harbors an MLL-r mutation, an MLL PTD mutation, a DNMT3A mutation, an FLT3 mutation, an IDH1 mutation, an IDH2 mutation, an NPM1 mutation, a splicing factor mutation, a TP53 mutation, a CEBPA mutation, an AML-ETO fusion mutation, or a combination thereof.

In some embodiments, the hematological cancer has or has been determined to have inv(3) mutation. In some embodiments, the hematological cancer has or has been determined to have 7/del(7q) mutation. In some embodiments, the hematological cancer has or has been determined to have SF3B1 mutation. In some embodiments, the hematological cancer has or has been determined to have MLLr mutation. In some embodiments, the hematological cancer has or has been determined to have RUNX1 mutation. In some embodiments, the hematological cancer has or has been determined to have ASXL1 mutation. In some embodiments, the hematological cancer has or has been determined to have JAK2 mutation. In some embodiments, the hematological cancer has or has been determined to have NRAS mutation. In some embodiments, the hematological cancer has or has been determined to have KRAS mutation. In some embodiments, the hematological cancer has or has been determined to have TP53 mutation. In some embodiments, the hematological cancer has or has been determined to have TET2 mutation. In some embodiments, the hematological cancer has or has been determined to have DNMT3 mutation.

In some embodiments, the invention provides a method of treating hematological cancer in a subject, wherein the hematologic cancer is acute myeloid leukemia or myelodysplastic syndrome.

In some embodiments, the invention provides a method of treating solid tumors in a subject, wherein the solid tumor is a tumor of the bladder, gastrointestinal tract, colon, breast, prostate, lung, kidney, liver, pancreas, ovary, head and neck, oral cavity, stomach, duodenum, small intestine, large intestine, anus, gall bladder, labium, nasopharynx, skin, uterus, male genital organ, urinary organs, and skin. Solid tumors of non-epithelial origin include sarcomas, brain tumors, and bone tumors.

In some embodiments, the solid tumor is melanoma, lung cancer, prostate cancer, or lymphoma.

In some embodiments, the melanoma is uveal melanoma, cutaneous melanoma

In some embodiments, the solid tumor is neuroendocrine, prostate cancer, and Immuno-oncology (IO).

In some embodiments, the lung cancer is non-small cell and small cell lung cancers.

In some embodiments, the method inhibits cancer cell invasion or migration in a subject having a high risk of cancer cell invasion or migration. In some embodiments, the method suppresses metastatic progression of cancer. In some embodiments, the method suppresses metastatic colonization of cancer. In some embodiments, the method slows the spread of a migrating cancer in a subject having a high risk of the spread of the migrating cancer. In some embodiments, the method reduced the rate of tumor seeding of a cancer in a subject having a high risk of tumor seeding of a cancer. In some embodiments, the method reduces or treats metastatic nodule-forming of a cancer in a subject having a high risk of metastatic nodule-forming of a cancer. In some embodiments, the method reduces metastatic risk of a cancer in a subject having a high metastatic risk. In some embodiments, the method treats metastatic cancer in a subject having a high metastatic risk. In some embodiments, the method reduces tumor heterogeneity.

Definitions

In this application, unless otherwise clear from context, (i) the term “a” may be understood to mean “at least one”; (ii) the term “or” may be understood to mean “and/or”; and (iii) the terms “comprising”

and “including” may be understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps.

As used herein, the term “differentiation dose regimen,” refers to a dosing regimen (i.e., a dosing level and frequency) of FHD 286, or a pharmaceutically acceptable salt thereof, that (a) increases the appearance of a marker of differentiation in one or more dedifferentiated cells of the cancer, (b) decreases the appearance of a marker of dedifferentiation in one or more dedifferentiated cells of the cancer, (c) induces cell arrest in one or more dedifferentiated cells of the cancer, or (d) induces apoptosis in in one or more dedifferentiated cells of the cancer. For example, in hematological cancers the differentiation dose regimen is sufficient to induce cell arrest and/or apoptosis in leukemic stem cells in a subject. For example, the differentiation dose regimen can be a dosing regimen (i.e. a dosing level and frequency) of FHD 286, or a pharmaceutically acceptable salt, that is sufficient to produce a $C_{average}$ plasma concentration level of at least 250 ng/mL (e.g., at least 250 ng/mL, 275 ng/mL, or 300 ng/mL) in a subject. For example, the average daily dose administered during the differentiation dose regimen can be greater than 7.5 mg to 22.5 mg.

As used herein, the term “maintenance dose regimen,” refers to a dosing regimen (i.e., a dosing level and frequency) of FHD 286, or a pharmaceutically acceptable salt thereof, following the administration of the differentiation dose regimen, that inhibits the proliferation of dedifferentiated cells of the cancer. For example, in hematological cancers, the maintenance dose regimen is insufficient to induce cell arrest and/or apoptosis in leukemic stem cells in a subject, but sufficient to reduce the number of blast cells. For example, in solid tumors, the maintenance dose is sufficient to reduce the level of cells with stem-cell like features e.g., increases DNA damage response. For example, the maintenance dose regimen can be a dosing regimen (i.e. a dosing level and frequency) of FHD 286, or a pharmaceutically acceptable salt, that is sufficient to produce a Caverage plasma concentration level of between about 80 ng/mL and about 225 ng/mL in a subject. For example, the average daily dose administered during the maintenance dose regimen can be 1.5 mg, 5 mg, or 7.5 mg.

As used herein, the term $C_{average}$ refers to AUC/t, wherein the AUC is the area under the curve and t is the dosing interval produced on average over 7 or more subjects for a given dosing level and frequency.

As used herein, the term “hematologic cancer,” refers to cancers that begin in blood-forming tissue, such as the bone marrow, or in the cells of the immune system, e.g., leukemias, lymphomas, and myelomas. Leukemias are cancers found in blood and bone marrow which are caused by rapid production of abnormal white blood cells. Lymphomas are cancers which affect the lymphatic system. Myelomas are cancers of the plasma cells. In most hematologic cancers, normal blood cell development is interrupted by uncontrolled growth of an abnormal type of blood cell. The abnormal blood cells prevent the blood from performing many of its functions. Hematologic cancers account for about 10% of all new cancer diagnoses. The 5-year relative survival rates for hematologic cancers range from about 50% to about 90%.

As used herein, the term “solid tumor,” refers to any cancer of body tissues other than blood, bone marrow, or the lymphatic system. Solid tumors can be further divided into those of epithelial cell origin and those of non-epithelial cell origin. Examples of epithelial cell solid tumors include tumors of the bladder, gastrointestinal tract, colon, breast, prostate, lung, kidney, liver, pancreas, ovary, head and neck,

oral cavity, stomach, duodenum, small intestine, large intestine, anus, gall bladder, labium, nasopharynx, skin, uterus, male genital organ, urinary organs, and skin. Solid tumors of non-epithelial origin include sarcomas, brain tumors, and bone tumors.

As used herein, the term “differentiation” refers to the process by which unspecialized ("not associated") or less specialized cells acquire the function of specialized cells, such as nerve cells or muscle cells.

As used herein, the term “differentiated cells” are cells, preferably stem or stem-like cells, that have been induced to differentiate are those that have transitioned to a more specialized ("differentiated") position within the lineage of a cell. For example, a differentiated cell has progressed through a differentiation pathway and continues to differentiate toward a specific cell type or part of a cell type under normal circumstances. This means that the stage has reached a stage where it cannot differentiate into another cell type under normal circumstances and cannot return to a cell type with a low degree of differentiation.

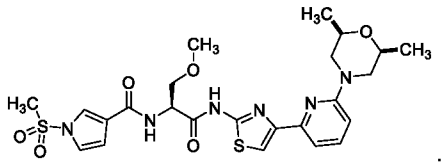
As used herein “dedifferentiation” refers to the process by which a cell returns to a less differentiated (less specialized) stage in the cell lineage. As used herein, the lineage of a cell defines the inheritance of the cell (i.e., which cell came from where and which cells can form). The cell lineage is the arrangement of cells within the genetic system of development and differentiation. Lineage-specific markers refer to characteristics that are specific to the cell phenotype of the genealogy of interest and can be used to assess the differentiation of unassociated cells into the genealogy of interest.

As used herein, the term “dedifferentiated cells” refer to cells that have returned to a less specialized position within the lineage of the cell.

As used herein, the term “marker of differentiation” refers to refers to gene expression, physiology, function within the organism, proliferative activity, or morphology of a cell that demonstrates a more specialized or later state of the cell. For example, the markers of differentiation may be CD19, CD24, CD38, CD87, CD133, CD166, CD90, EpCAM, ALDH, Nanog, and Oct-3/4. For example, in hematological cancers, the markers for differentiation are CD11b Gr-1, CD33, CD123, CL-L-1, TIM3, ALDH, Nanog, Oct-3/4, and Sox2. For example. in breast cancer, the marker of differentiation may be CD24, CD29, CD49f, CD61, CD70, CD90, CD133, CXCR4, EpCAM, LGR5, ProC-R, ALDH and BMI-1, Nanog, Notch, Oct-3/4, Sox2, Wnt/B-catenin. For example. in melanoma, the marker of differentiation may be NGFR (CD271), ABCB5 and CD20. For example. in prostate cancer, the marker of differentiation may be and CD133. For example. in lung cancer, the marker of differentiation may be CD87, CD133, CD166, CD90, EpCAM, ALDH, Nanog, Oct-3/4.

As used herein, the term “marker of dedifferentiation” refers to gene expression, physiology, function within the organism, proliferative activity, or morphology of a cell that demonstrates a less specialized or earlier state of the cell. For example, the markers of dedifferentiation may be CD44, KIT, CD34, CD117, EVI1, TP63, KRT5, KRT14, PROS1, PRAME, GDF15, and FOXP3.

As used herein, the term “FHD 286” refers to N-(1-((4-(6-(2,6-dimethylmorpholino)pyridin-2-yl)thiazol-2-yl)amino)-3-methoxy-1-oxopropan-2-yl)-1-(methylsulfonyl)-1H-pyrrole-3-carboxamide. As used herein, FHD 286 has the structure:



The term “cancer” refers to a condition caused by the proliferation of malignant neoplastic cells, such as tumors, neoplasms, carcinomas, sarcomas, leukemias, and lymphomas.

As used herein, “metastatic cancer” refers to a tumor or cancer in which the cancer cells forming the tumor have a high potential to or have begun to, metastasize, or spread from one location to another location or locations within a subject, via the lymphatic system or via haematogenous spread, for example, creating secondary tumors within the subject. Such metastatic behavior may be indicative of malignant tumors. In some cases, metastatic behavior may be associated with an increase in cell migration and/or invasion behavior of the tumor cells.

Examples of cancers that can be defined as metastatic include, but are not limited to, lung cancer (e.g., non-small cell lung cancer), breast cancer, ovarian cancer, colorectal cancer, biliary tract cancer, bladder cancer, brain cancer including glioblastomas and medulloblastomas, cervical cancer, choriocarcinoma, endometrial cancer, esophageal cancer, gastric cancer, hematological neoplasms, multiple myeloma, leukemia, intraepithelial neoplasms, liver cancer, lymphomas, neuroblastomas, oral cancer, pancreatic cancer, prostate cancer, sarcoma, skin cancer including melanoma, basocellular cancer, squamous cell cancer, testicular cancer, stromal tumors, germ cell tumors, thyroid cancer, and renal cancer.

“Non-metastatic cell migration cancer” as used herein refers to cancers that do not migrate via the lymphatic system or via haematogenous spread.

“Progression-free survival” as used herein, refers to the length of time during and after medication or treatment during which the disease being treated (e.g., cancer) does not get worse.

“Proliferation” as used in this application involves reproduction or multiplication of similar forms (cells) due to constituting (cellular) elements.

As used herein, “slowing the spread of metastasis” refers to reducing or stopping the formation of new loci; or reducing, stopping, or reversing the tumor load.

As used herein, the term “administration” refers to the administration of a composition (e.g., a compound or a preparation that includes a compound as described herein) to a subject or system. Administration to an animal subject (e.g., to a human) may be by any appropriate route. For example, in some embodiments, administration may be bronchial (including by bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intratumoral, intravenous, intraventricular, mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal, and vitreal.

As used herein, a “combination therapy” or “administered in combination” means that two (or more) different agents or treatments are administered to a subject as part of a defined treatment regimen for a particular disease or condition. The treatment regimen defines the doses and periodicity of administration of each agent such that the effects of the separate agents on the subject overlap. In some embodiments, the delivery of the two or more agents is simultaneous or concurrent and the agents may

be co-formulated. In some embodiments, the two or more agents are not co-formulated and are administered in a sequential manner as part of a prescribed regimen. In some embodiments, administration of two or more agents or treatments in combination is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one agent or treatment delivered alone or in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive (e.g., synergistic). Sequential or substantially simultaneous administration of each therapeutic agent can be affected by any appropriate route including, but not limited to, oral routes, intravenous routes, intramuscular routes, and direct absorption through mucous membrane tissues. The therapeutic agents can be administered by the same route or by different routes. For example, a first therapeutic agent of the combination may be administered by intravenous injection while a second therapeutic agent of the combination may be administered orally.

The term “pharmaceutical composition,” as used herein, represents a composition containing a compound described herein formulated with a pharmaceutically acceptable excipient and appropriate for administration to a mammal, for example a human. Typically, a pharmaceutical composition is manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment of disease in a mammal. Pharmaceutical compositions can be formulated, for example, for oral administration in unit dosage form (e.g., a tablet, capsule, caplet, gelcap, or syrup); for topical administration (e.g., as a cream, gel, lotion, or ointment); for intravenous administration (e.g., as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use); or in any other pharmaceutically acceptable formulation.

A “pharmaceutically acceptable excipient,” as used herein, refers to any ingredient other than the compounds described herein (for example, a vehicle capable of suspending or dissolving the active compound) and having the properties of being substantially nontoxic and non-inflammatory in a patient. Excipients may include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspending or dispersing agents, sweeteners, and waters of hydration.

As used herein, the term “pharmaceutically acceptable salt” means any pharmaceutically acceptable salt of a compound described herein. Pharmaceutically acceptable salts of any of the compounds described herein may include those that are within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and animals without undue toxicity, irritation, allergic response and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, pharmaceutically acceptable salts are described in: Berge et al., J. Pharmaceutical Sciences 66:1-19, 1977 and in Pharmaceutical Salts: Properties, Selection, and Use, (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 2008. The salts can be prepared in situ during the final isolation and purification of the compounds described herein or separately, e.g., by reacting to a free base group with a suitable organic acid. The compounds of the invention may have ionizable groups so as to be capable of preparation as pharmaceutically acceptable salts. These salts may be, e.g., acid addition salts involving inorganic or organic acids or the salts may, in the case of acidic forms of the compounds of the invention be prepared from inorganic or organic bases. Frequently, the compounds are prepared or used as pharmaceutically acceptable salts prepared as addition products of pharmaceutically acceptable

acids or bases. Suitable pharmaceutically acceptable acids and bases and methods for preparation of the appropriate salts are well-known in the art. Salts may be prepared from pharmaceutically acceptable non-toxic acids and bases including inorganic and organic acids and bases.

As used herein, the term “progression-free survival” as used herein, refers to the length of time during and after medication or treatment during which the disease being treated (e.g., cancer) does not get worse. The combination therapies of the invention can increase the likelihood of progression-free survival in a subject.

As used herein, the term “proliferation” as used in this application involves reproduction or multiplication of similar forms (cells) due to constituting (cellular) elements. The combination therapies of the invention can decrease proliferation of cancer cells.

As used herein, the term “subject” refers to any organism to which a composition in accordance with the disclosure may be administered, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include any animal (e.g., mammals such as mice, rats, rabbits, non-human primates, and humans). A subject may seek or be in need of treatment, require treatment, be receiving treatment, be receiving treatment in the future, or be a human or animal who is under care by a trained professional for a particular disease or condition.

As used herein, the terms "treat," "treated," or "treating" mean therapeutic treatment or any measures whose object is to slow down (lessen) an undesired physiological condition, disorder, or disease, or obtain beneficial or desired clinical results. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of the extent of a condition, disorder, or disease; stabilized (i.e., not worsening) state of condition, disorder, or disease; delay in onset or slowing of condition, disorder, or disease progression; amelioration of the condition, disorder, or disease state or remission (whether partial or total); an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient; or enhancement or improvement of condition, disorder, or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment. In the context of treating cancer, treatment may include slowing the spread of metastasis and/or extending progression-free survival in a population of treated subjects as compared to a population of untreated subjects. Compounds of the disclosure may also be used to “prophylactically treat” or “prevent” a disorder, for example, in a subject at increased risk of developing the disorder.

As used herein, “average daily dose” refers to the administered dose, in milligrams, of FHD 286 or a pharmaceutically acceptable salt. The average daily dose is calculated from the instructed regimen. For example, 10 mg daily is an average daily dose of 10 mg/day, 10 mg on day 1 followed by 6 days of no doses is an average daily dose of 1.43 mg/day, 10 mg dose on day 1 followed by 13 days of no doses is an average daily dose of 0.71 mg/day, 10 mg twice weekly is an average daily dose of 2.85 mg/day (20 mg/7 days); 10 mg weekly followed by 20 mg weekly followed by 20 mg twice weekly is an average daily dose in week 1 of 1.43 mg/day (10 mg/7 days), in week 2 of 2.86 mg/day (20 mg/7 days), and in week 3 of 5.71 mg/day (40 mg/7 days). For regimens of indefinite intervals, the average daily dose is calculated from the average of the high and low values. For example, a regimen that calls for 15 mg every seven to ten days has an average daily dose of 1.82 mg/day {(15 mg/7 days+15 mg/10 days)/2}. Average daily doses are in the range of from, for example, 1 to 2.5 mg/day, 1 to 5 mg/day, 1 to 7.5 mg/day, 1 to 10

mg/day, or 10 to 12.5 mg/day, 10 to 15 mg/day, 10 to 17.5 mg/day, 10 to 20 mg/day, or 10 to 22.5 mg/day of FHD 286 or a pharmaceutically acceptable salt.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Methods and materials are described herein for use in the present disclosure; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

The details of one or more embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

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

Fig. 1. shows the study design.

Fig. 2 shows a series of graphs depicting the count of total cells and % of CD11b+ cells on Day 10 as described in Example 1.

Fig. 3 shows a series of graphs depicting the cells with CD11b+/-, BRG1+/-, and/or Ki67+/- markers in the population on Day 10 as described in Example 1.

Fig. 4 shows a series of graphs depicting the change in % of CD11b+ cells on Day 10, Day 14 and Day 17 as described in Example 1.

Fig. 5 shows a series of graphs depicting the % of CD11b cells on Day 7, Day 14 and Day 17 after a Day 7 or Day 10 washout as described in Example 2.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure features a differentiation and maintenance dosing regimen of FHD 286 for the treatment of cancer, including AML, related hematological cancers and solid tumors. This therapy can reduce side effects, improve outcomes, and/or reduce the dosing burden on the patient. For example, using the methods described herein the risk of developing differentiation syndrome can be reduced in the subject relative to treatment with the FHD 286, or a pharmaceutically acceptable salt thereof. In some embodiments, the methods of the present disclosure result in one or more (e.g., two or more, three or more, four or more) of: (a) decrease blast count, (b) normal neutrophil counts, (c) normal platelet counts, (d) a bone marrow biopsy which reveals no clusters or collections of blast cells, (e) decreased tumor recurrence (f) increased survival of subject, (g) increased progression free survival of subject.

This invention is based in, at least in part, on the discovery that the dedifferentiated cell population, a subgroup of cancer cells, is responsible for the chemoresistance and cancer relapse, as it has ability to self-renew and to differentiate into the heterogeneous lineages of cancer cells in response to

chemotherapeutic agents. Dedifferentiated cells are also able to induce cell cycle arrest (quiescent state) that support their ability to become resistant to chemo- and radiotherapy. Common chemotherapeutic agents target the proliferating cells to lead their apoptosis, as mentioned previously. Although successful cancer therapy abolishes the bulk of proliferating tumor cells, a subset of remaining dedifferentiated cells can survive and promote cancer relapse due to their ability to establish higher invasiveness and chemoresistance. The methods of the invention provide a method for treating the dedifferentiated cells of a cancer to address a limitation of many anti-cancer therapies.

Treating cancer can result in an increase in the average survival time of a population of subjects treated according to the present invention in comparison to a population of untreated subjects. For example, the average survival time is increased by more than 30 days (more than 60 days, 90 days, or 120 days). An increase in average survival time of a population may be measured by any reproducible means. An increase in average survival time of a population may be measured, for example, by calculating for a population the average length of survival following initiation of treatment with the compound of the invention. An increase in average survival time of a population may also be measured, for example, by calculating for a population the average length of survival following completion of a first round of treatment with a pharmaceutically acceptable salt of the invention.

Treating cancer can also result in a decrease in the mortality rate of a population of treated subjects in comparison to an untreated population. For example, the mortality rate is decreased by more than 2% (e.g., more than 5%, 10%, or 25%). A decrease in the mortality rate of a population of treated subjects may be measured by any reproducible means, for example, by calculating for a population the average number of disease-related deaths per unit time following initiation of treatment with a pharmaceutically acceptable salt of the invention. A decrease in the mortality rate of a population may also be measured, for example, by calculating for a population the average number of disease-related deaths per unit time following completion of a first round of treatment with a pharmaceutically acceptable salt of the invention.

Exemplary hematological cancers that may be treated by the invention include, but are not limited to multiple myeloma, large cell lymphoma, acute T-cell leukemia, acute myeloid leukemia, myelodysplastic syndrome, immunoglobulin A lambda myeloma, diffuse mixed histiocytic and lymphocytic lymphoma, B-cell lymphoma, acute lymphoblastic leukemia, diffuse large cell lymphoma, or non-Hodgkin's lymphoma.

The hematologic cancer can be characterized as having a inv(3) mutation, a 7/del(7q) mutation, a SF3B1 mutation, a MLLr mutation, a RUNX1 mutation, an ASXL1 mutation, a JAK2 mutation, a NRAS mutation, a KRAS mutation, a TP53 mutation, a TET2 mutation, and/or a DNMT3A mutation.

Exemplary solid tumor cancers that may be treated by the invention include, but are not limited to melanoma, small cell lung cancer, non-small cell lung cancer, or prostate cancer.

FHD 286

Methods for synthesizing FHD 286 are described in International Application No. PCT/US2021/015876, the content of which is incorporated herein by reference in its entirety. Methods of treatment of FHD 286 are described in International Application No. PCT/US2021/015876, the content of which is incorporated herein by reference in its entirety. Methods of treating cancer by FHD 286 are

described in International Application No. PCT/US23/17829, the content of which is incorporated herein by reference in its entirety.

Differentiation dose regimen

The differentiation dose regimen is typically an initial dose regimen (i.e., in a subject that is treatment naïve or has not received a dose of FHD 286 for a period of at least 30 days) that comprises a higher dose than subsequent doses. In any of the embodiments, the differentiation dose regimen is a dosing regimen (i.e. a dosing level and frequency) of FHD 286, or a pharmaceutically acceptable salt, that (a) increases the appearance of a marker of differentiation in one or more dedifferentiated cells of the cancer, (b) decreases the appearance of a marker of dedifferentiation in one or more dedifferentiated cells of the cancer, (c) induces cell arrest in one or more dedifferentiated cells of the cancer, or (d) induces apoptosis in in one or more dedifferentiated cells of the cancer. For example, the differentiation dose regimen can be a dosing regimen (i.e. a dosing level and frequency) of FHD 286, or a pharmaceutically acceptable salt, that is sufficient to produce a Caverage plasma concentration level of at least 250 ng/mL (e.g., at least 250 ng/mL, 275 ng/mL, or 300 ng/mL) in a subject. The differentiation doses are administered daily, once a week, 2 to 7 times per week, once in 2 weeks, 2 to 14 times in 2 weeks, once in three weeks, 2 to 21 times in three weeks, once in four weeks, 2 to 28 times in four weeks, or once every 28 days. It can include but is not limited to administration of unit dosage forms on any dosing schedule contemplated herein, including but not limited to times. It is contemplated that, within the scope of this disclosure, a drug holiday may be incorporated into the differentiation dose administration period. Such a drug holiday can occur immediately after administration of a differentiation dose, the period of the differentiation dose administration may be referred to as the “differentiation phase” of the treatment period.

Maintenance dose regimen

The maintenance dose regimen refers to a subsequent dose regimen following a differentiation dose regimen of FHD 286 or pretreatment with a cytotoxic agent, and is administered subsequent to the differentiation dose or the pretreatment with a cytotoxic agent. One skilled in the art will appreciate that the dosage form for maintenance doses may differ from that used for differentiation doses. The maintenance dose comprises a lower dose than differentiation or initial doses. In any of the embodiments, the maintenance dose regimen is a dosing regimen (i.e. a dosing level and frequency) of FHD 286, or a pharmaceutically acceptable salt, that following the administration of the differentiation dose regimen, that inhibits the proliferation of dedifferentiated cells of the cancer. For example, the maintenance dose regimen can be a dosing regimen (i.e. a dosing level and frequency) of FHD 286, or a pharmaceutically acceptable salt, that is sufficient to produce a Caverage plasma concentration level of between about 80 ng/mL and about 225 ng/mL in a subject. In any of the embodiments disclosed herein, maintenance doses are administered daily, once a week, 2 to 7 times per week, once in 2 weeks, 2 to 14 times in 2 weeks, once in three weeks, 2 to 21 times in three weeks, once in four weeks, 2 to 28 times in four weeks, or once every 28 days. In any of the embodiments disclosed herein, maintenance doses are administered over a period of at least one month, two months, three months, four months, six months, 1 year, 2 years, 5 years, 10 years or 20 years. It can include but is not limited to administration of unit dosage forms on any dosing schedule contemplated herein, including but not limited to times. It is contemplated that, within the scope of this disclosure, a drug holiday may be incorporated into the differentiation dose administration period. Such a drug holiday can occur immediately after administration of a differentiation dose, or at any time during administration of a maintenance dose. The period of the maintenance dose administration may be referred to as the “maintenance phase” of the treatment period.

Additional details are provided in the Examples.

Differentiation and Dedifferentiation of Cancer Cells

There are multiple levels of heterogeneity associated with cancer, and this heterogeneity is one of the hallmarks of cancers arising in several organs. Genetic heterogeneity in the majority of the cancers is reflected by genome instability, and in addition to these genetic alterations, the state of the cell may be changed epigenetically. Phenotypic heterogeneity refers to the diverse functional properties and expression of different lineage markers that tumor cells can adopt along cancer progression. Based on cell surface markers, it is possible to identify distinct subpopulations of neoplastic cells within the same tumors, suggesting that irrespective of their genetic alterations, cancer cells exist in different states of differentiation (see, e.g., Visvader et al., Cancer stem cells: current status and evolving complexities. Cell Stem Cell 10: 717–728 (2012)). This heterogeneity is referred to as intratumor heterogeneity, to explain the variations found within individual tumors, and intertumoral heterogeneity refers to the molecular differences that occur between tumors initiated in the same organ, which allows the classification of these tumors in different subtypes, and which may (in some circumstances) represent biologically distinct disease entities (see, e.g., Visvader JE Cells of origin in cancer. Nature 469: 314–322 (2011)).

Cancer stem cells are the cells within a tumor that possess the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor. CSCs are thus a biologically unique subpopulation of cells that can perpetuate indefinitely as opposed to the bulk of cells that reside in

the tumor, and are mostly insensitive to currently used cancer therapies. The CSCs are a subpopulation of cells that sustain malignant growth by means of their ability to self-renew and the possibility to give rise to progeny with self-limited proliferative capacity. This suggests a hierarchical organization where CSCs are responsible for the generation of the heterogeneity found within tumors. Although CSCs exhibit the stem cell properties of self-renewal and differentiation, they do not necessarily originate from the transformation of normal tissue stem cells.

Previous studies suggest that not all cancers strictly conform to the unidirectional hierarchical CSC model, and entertain the possibility of "tumor cell plasticity", where non-CSC can dedifferentiate and acquire CSC-like properties under certain conditions as demonstrated by the examples described below of glioblastoma, intestinal cancer, metastatic cancer, and invasive tumors, among others.

Glioblastoma

Glioblastoma (GBM), the most common and aggressive subtype of the malignant gliomas, is characterized by intense proliferation, invasion, and intratumor heterogeneity. It has previously been demonstrated that the combined loss of $p16^{INK4a}$ and $p19^{ARF}$ enables mature astrocyte dedifferentiation in response to EGFR activation (see, e.g., Bachoo et al., Epidermal growth factor receptor and Ink4a/Arf: convergent mechanisms governing terminal differentiation and transformation along the neural stem cell to astrocyte axis. Cancer Cell 1: 269–277 (2002)). The identification of TUJ1-positive neurons in the tumors originating from the transformed astrocytes suggested that dedifferentiation may be so complete as to generate a pluripotent cell with the potential to make neurons as well as glia. It has also been shown that GBM can originate from a variety of cells in the brain, including terminally differentiated cortical astrocytes and neurons (See, e.g., Friedmann-Morvinski et al., Dedifferentiation of neurons and astrocytes by oncogenes can induce gliomas in mice. Science 338: 1080–1084 (2012)). Transduction by oncogenic Cre-inducible lentiviruses in the cortex of synapsinI-Cre or GFAP-Cre transgenic mice, which drive the expression of Cre specifically in neurons and glial cells, respectively, induced the formation of gliomas. These tumors mostly express markers of progenitor/neural stem cells, nestin and Sox2. It has also been observed that at early stages, differentiation markers are progressively diminished, while nestin, a marker of NSC, undetectable a few days after transduction, increases significantly with tumor progression. This suggests that oncogenic-induced dedifferentiation of mature cells in the brain to a stem-/progenitor-like state leads to heterogeneous glioma tumors. The genetically acquired plasticity of these cells allows progression and maintenance of this aggressive tumor and even formation of its own blood vessels by transdifferentiation (see, e.g., Soda et al. Transdifferentiation of glioblastoma cells into vascular endothelial cells. Proc Natl Acad Sci USA 108: 4274–4280 (2011)). Glioblastoma tumors induced by oncogenic lentivirus either in neurons or in glia in the cortex initially express differentiation markers (e.g., Tuj1 and GFAP, respectively), but as tumor progresses, these markers decrease and stem/progenitor markers become predominantly expressed (like nestin and Sox2) (See, e.g., Friedmann-Morvinski et al., Dedifferentiation of neurons and astrocytes by oncogenes can induce gliomas in mice. Science 338: 1080–1084 (2012)).

Intestinal Cancer

The formation of CSCs from non-CSCs has also been found in tumors formed from intestinal epithelial cells (IEC). For example, the combination of an oncogenic hit like Kras and the activation of NF-κB induces the stabilization of β-catenin and thereof the activation of the β-catenin/tcf transcription complex, leading to the conversion of non-stem IEC into IEC with stem cell properties (see, e.g., Schwitalla et al., Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. Cell 152: 25–38 (2013)). Both Kras and TNF-α-dependent NF-κB activation enhances β-catenin/Tcf-mediated transcriptional activity and induces dedifferentiation of non-stem IEC into tumor-initiating cells.

Hematological Cancer

Hematologic cancers, also known as blood cancers, are cancers that begin in blood-forming tissue, such as the bone marrow, or in the cells of the immune system, e.g., leukemias, lymphomas, and myelomas. Leukemias are cancers found in blood and bone marrow which are caused by rapid production of abnormal white blood cells. Lymphomas are cancers which affect the lymphatic system. Myelomas are cancers of the plasma cells. In most hematologic cancers, normal blood cell development is interrupted by uncontrolled growth of an abnormal type of blood cell. The abnormal blood cells prevent the blood from performing many of its functions. Hematologic cancers account for about 10% of all new cancer diagnoses. The 5-year relative survival rates for hematologic cancers range from about 50% to about 90%.

Acute myeloid leukemia (AML), also known as acute myelogenous leukemia or acute non-lymphocytic leukemia (ANLL), is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. AML is the most common acute leukemia affecting adults, and its incidence increases with age. Although AML is a relatively rare disease (accounting for approximately 1.2% of cancer deaths in the U.S.) its incidence is expected to increase as the population ages.

The symptoms of AML are caused by replacement of normal bone marrow with leukemic cells, which causes a drop in red blood cells, platelets and normal white blood cells. These symptoms include fatigue, shortness of breath, easy bruising and bleeding, and increased risk of infection. Several risk factors and chromosomal abnormalities have been identified, but the specific cause of AML is unclear at this time. As an acute leukemia, AML progresses rapidly and is typically fatal within weeks or months if left untreated.

One hallmark of AML is that the myeloid leukemic blast is arrested at an early stage of differentiation. Prior to the development of fine karyotyping and genetic analysis, morphologic hallmarks of immaturity were used to classify a patient's disease histologically (e.g. the 1976 FAB classification scheme). The recognition that leukemic blasts seemed frozen at an immature stage of differentiation suggested that new therapies might be directed at promoting differentiation rather than simply killing leukemic cells.

Metastases

The implications of tumor plasticity can go beyond the primary tumor and play an important role in tumor metastasis. It has been suggested that CSCs are endowed with multiple traits that are required for most of the steps related to the invasion–metastasis cascade and therefore might be key players in metastatic tumors. (see, e.g., Thiery et al., Epithelial-mesenchymal transitions in development and disease. Cell 139: 871–890 (2009)).

In general, the treatment of tumors, which have resident cancer stem cells, with antiproliferative agents will not be very effective, leading to recurrence, unless the stem cells are also eliminated. Using the methods of the invention such stem cells are reduced and/or eliminated for forcing the cancer stem cells to undergo differentiation.

Markers of Differentiation

Differentiated cells can be identified by specific markers of normal stem cells which are commonly used for isolating cells from solid and hematological tumors. Several cell surface markers have been verified to identify cancer-enriched populations, such as CD133, CD24, EpCAM (epithelial cell adhesion molecule), THY1, ABCB5 (ATP-binding cassette B5), and CD200. Additionally, certain intracellular proteins also have been used as differentiation markers, such as aldehyde dehydrogenase 1 (ALDH1) which is used to characterize differentiated cells in many types of cancer such as leukemia, breast, colon, liver, lung, pancreas, etc. The usage of cell surface markers as differentiation markers might differ from each cancer types depending on their characteristics and phenotypes. For example, the markers of differentiation may be CD19, CD24, CD38, CD87, CD133, CD166, CD90, EpCAM, ALDH, Nanog, and Oct-3/4. For example, in hematological cancers, the markers for differentiation are CD11b Gr-1, CD33, CD34+, CD38-, CD123+, CD123, CL-L-1, TIM3, ALDH, Nanog, Oct-3/4, and Sox2. For example. in breast cancer, the marker of differentiation may be CD24, CD29, CD49f, CD61, CD70, CD90, CD133, CXCR4, EpCAM, ABCG2, ESA, LGR5, ProC-R, ALDH and BMI-1, Nanog, Notch, Oct-3/4, Sox2, Wnt/B-catenin. For example. in melanoma, the marker of differentiation may be ABCB5, CD20, NGFR (CD271), ABCB5 and CD20. For example. in prostate cancer, the marker of differentiation may be $\alpha 2\beta 1$, ABCG2, ALDH, and CD133. For example. in lung cancer, the marker of differentiation may be ABCG2, ALDH, CD87, SP, CD87, CD133, CD166, CD90, EpCAM, ALDH, Nanog, Oct-3/4.

Pharmaceutical Compositions

The compounds of the present disclosure may be formulated into pharmaceutical compositions for administration to human subjects in a biologically compatible form suitable for administration in vivo. Pharmaceutical compositions typically include an active agent as described herein and a physiologically acceptable excipient (e.g., a pharmaceutically acceptable excipient). Formulation principles for the compounds disclosed herein may be those described, e.g., in WO 2020/160180, the disclosure of which is incorporated by reference herein in its entirety.

The compounds of the disclosure may be administered, for example, by oral, parenteral, buccal, sublingual, nasal, rectal, patch, pump, or transdermal administration and the pharmaceutical compositions formulated accordingly. Parenteral administration includes intravenous, intraperitoneal,

subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, rectal, and topical modes of administration. Parenteral administration may be by continuous infusion over a selected period of time. Preferably, the compound is administered orally.

Suitable pharmaceutical carriers, as well as pharmaceutical necessities for use in pharmaceutical formulations, are described in Remington: The Science and Practice of Pharmacy, 21st Ed., Gennaro, Ed., Lippencott Williams & Wilkins (2005), a well-known reference text in this field, and in the USP/NF (United States Pharmacopeia and the National Formulary).

In one embodiment, the FHD 286 may be formulated into a unit dosage form for oral administration (e.g., a capsule) as described in Table 1.

Table 1. Formulation of FHD 286

Component	Function	2.5 mg Capsule		20 mg Capsule	
		Amount per Capsule (mg)	Content (%)	Amount per Capsule (mg)	Content (%)
API (FHD 286)	Active ingredient	2.50	2.63	20.00	17.39
Microcrystalline cellulose	Filler	48.24	50.78	48.20	41.91
Mannitol	Filler	36.18	38.08	36.95	32.13
Croscarmellose Sodium	Disintegrant	4.75	5.00	5.80	5.04
Sodium lauryl sulfate	Wetting agent	0.95	1.00	1.15	1.00
Colloidal silicon dioxide	Glidant	1.90	2.00	2.30	2.00
Magnesium stearate	Lubricant	0.48	0.51	0.60	0.52
Size 4, Swedish Orange HPMC Capsules	Capsule Shell	1 capsule	-	-	-
Size 3, Blue Green HPMC Capsules	Capsule Shell	-	-	1 capsule	-
Total		95.00	100	115.00	100

Examples

The cell culture method, flow cytometry method and flow cytometry analysis methods are relevant to the examples below.

Cell culture method

AML cell lines were maintained in vitro as suspension cells in the media listed (Table 2) and subcultured every 3 or 4 days by diluting to 0.5×10^6 cells/mL or 0.3×10^6 cells/mL, respectively, in fresh growth medium. Cells were discarded upon reaching passage 15.

CD34+ cells were isolated from primary cell samples (Proteogenex; Inglewood, CA) using EasySep Human CD34 Positive Selection Kit II (STEMCELL Technologies [STEMCELL]; Vancouver, BC, Canada) per manufacturer protocol. The separation step was repeated a total of 4 times. The cells were resuspended in 1 mL of Stem Cell Medium and seeded at 0.5×10^6 cells/mL for immediate use in the 7-day differentiation assay.

All cells were maintained at 37°C in an atmosphere of 5% CO_2.

Table 2: AML Cells and Culture Conditions

Cell Line	Source	Cell Culture Conditions and Reagents
EOL-1	DSMZ	RPMI 1640 (Thermo Fisher #61870036) + 10% FBS (Omega #FB-16)
HL60	ATCC	IMDM (Thermo Fisher #31980030) + 20% FBS
HEL92.1.7	ATCC	RPMI 1640 + 10% FBS
MOLM13	DSMZ	RPMI 1640 + 10% FBS
MV-4-11	ATCC	IMDM + 10% FBS
OCI-AML2	DSMZ	MEM (Thermo Fisher #11095080) + 10% FBS
AML852L	Proteogenex	Stem Cell Medium: • StemSpan SFEM II (STEMCELL #09605) • 1× StemSpan Human CD34 Expansion Supplement (STEMCELL #02691) • 1% pen/strep (Thermo Fisher #15140148) • 20 ng/mL human rG-CSF (STEMCELL #78012.1) • 5 ng/mL human rGM-CSF (STEMCELL #78140)

Abbreviations: AML = acute myeloid leukemia; FBS = fetal bovine serum; IMDM = Iscove's Modified Dulbecco's medium; MEM = minimum essential medium; pen/strep = penicillin/streptomycin; rG-CSF = recombinant granulocyte colony-stimulating factor; rGM-CSF = recombinant granulocyte macrophage stimulating factor; RPMI = Roswell Park Memorial Institute.

Flow Cytometry Method

On the day of analysis treated cells were transferred to v-bottomed plates. Medium and test article were removed by centrifuging the plate at 300 RCF for 5 minutes and discarding the supernatant. The plate was washed by adding 200 µL/well of cold FACS buffer, centrifuged at 400 RCF for 5 minutes, and then the supernatant was discarded. Cells were resuspended in viability stain diluted 1:1000 in phosphate-buffered saline (PBS) and incubated for 15 minutes on ice in the dark. The stain was removed by centrifugation and then the plate was washed with FACS buffer as before. The plate was blocked with 50 µL/well of Fc block diluted 1:20 in FACS buffer for 15 minutes on ice in the dark. The Fc block was removed by centrifugation and then the plate was washed with FACS buffer as before. Cells were resuspended in 50 µL surface antibody cocktail diluted in FACS buffer and incubated for 30 minutes on ice in the dark. The antibody cocktail was removed by centrifugation and then the plate was washed with FACS buffer as before. Cells were resuspended in Fix/Perm at 200 µL/well and incubated for 30 minutes on ice in the dark. The Fix/Perm was removed by centrifugation, and the plate was washed as before with 200 µL/well Perm buffer. Cells were resuspended in 50 µL/well of intracellular antibody cocktail diluted in Perm buffer and incubated for 30 minutes on ice in the dark. The Perm buffer was removed by centrifugation, and then the plate was washed as before with 200 µL/well Perm buffer. Cells were resuspended in 100 µL/well FACS buffer and analyzed on the flow cytometer. Compensation was performed per manufacturer protocols. Number of events collected were ≥ 50,000 for the 7-day assay; 200,000 for the 14-day assays; and 150,000 for the 17-day assay. For the combination experiments, constant sample volume was analyzed to enable comparison of number of events between samples. Antibodies and key flow cytometry reagents used in the study are summarized in Table 3.

Table 3: Antibodies and Key Reagents Used for Flow Cytometric Analysis

Antibody/Reagent	Fluorophore	Dilution	Source	Catalog Number
BCL-2 (clone BCL-2/100)	PE-CF594	1:20	BD Biosciences	563601
BRG1 (clone EPNCIR111A)	AF488	1:200	Abcam	ab196314
CD11b (clone ICRF44)	SB780	1:20	Thermo Fisher	78-0118-42
Ki67 (clone 20Raj1)	APC	1:20	Thermo Fisher	17-5699-42
eBioscience Fixable Viability Dye eFluor 506	eFluor 506	1:1000	Thermo Fisher	65-0866-14
Fc Block: Human TruStain FcX	—	1:20	BioLegend	422302
AbC Total Antibody Compensation Bead Kit	—	—	Thermo Fisher	A10497
ArC Amine Reactive Compensation Bead Kit	—	—	Thermo Fisher	A10346
ViaStain AOPI Staining Solution	—	—	Nexcelom Bioscience	CS2-0106-5ML
Fix/Perm and Perm buffer: eBioscience Foxp3/Transcription Factor Staining Buffer Set	—	—	Thermo Fisher	00-5523-00
FACS Buffer: • 2% heat-inactivated FBS + • PBS without calcium and magnesium	—	—	• Omega • Thermo Fisher	• FB-16 • 10010

Abbreviations: AF = Alexa Fluor; APC = allophycocyanin; CF = cyanine-based fluorescence; FBS = fetal bovine serum; PBS = phosphate-buffered saline; PE = phycoerythrin; SB = Super Bright 780.

Flow Cytometry Analysis Method

Flow cytometry data were acquired using CytExpert (v2.5; Beckman Coulter; Brea, CA), and FlowJo (v10.7; Becton Dickinson; Franklin Lakes, NJ) was used for data analysis. First, cell gates were determined by forward scatter (FSC) versus side scatter (SSC) and debris was excluded. Single cells were then identified using SSC-Area and SSC-Height, and dead cells staining for viability dye were gated out.

For CD11b, thresholds for positive and negative marker expression were defined using an unstained control sample. BRG1 and Ki67 expression levels were evaluated by 2-parameter plots against CD11b. Cell populations of interest were divided into subpopulations of high or low marker expression by setting a visual threshold. For BCL-2, mean fluorescence intensity was calculated using FlowJo software.

Examples

Example 1. Cancer cell lines are treated with FHD 286

Procedure: Cancer Cell lines were treated were cultures in the presence of FHD 286 (20nM). After Day 10, the cells were sorted into groups of CD11B+ cells, CD11B- cells, and non-isolated cells. (See study design, Figure 1). In order to identify genetic biomarkers, each batch of cells were then stained with BV785-CD11b, FTIC-BRG1 and APC-Ki67 and the expression levels were measured by flow cytometry. (See Figure 2-3), The batches of cells were treated with FHD 286 (ranging from 0 nM to 20 nM) for a total of 17 days. At Day 14 and Day 17, each batch of cells were then stained with BV785-CD11b and the expression levels were measured by flow cytometry. (See Figure 4).

Results: 5nM dose after the pre-treatment of cells with 20 nM FHD 286 is sufficient to maintain the CD11b+ expression in CD11b+ cells. The 2.5 nM dose will slowly decrease the CD11b+ in the CD11b+ population. The 10 nM dose slowly increases the CD11b+ in the CD11b population. All doses of FHD 286 increases the CD11b+ in the CD11b- population.

Example 2. Cancer cell lines are treated with FHD 286 with washout on Day 7 and Day 10

Procedure: CD11b+ and CD11b- cell lines were treated were cultures in the presence of FHD 286 (20nM). On Day 7 or Day 10, the cells were washed out. Then the cells were treated with either 20 nM FHD 286 or DMSO. The cells were treated until Day 17. The cells were treated with BV785-CD11b to identify the %CD11b. (See Figure 5).

Results: The washout of FHD 286 on Day 7 or Day 10, demonstrates that the FHD 286 continues to maintain an effect even after the compound is present. The comparison between the Day 7 and Day 10 washout demonstrates that the longer the presence of FHD 286 is, the longer the effect is seen after the washout.

Example 3. Differentiation dose regimen followed by maintenance dose regimen of FHD 286 for the treatment of cancer.

Subjects with cancer are treated with a differentiation dose of FHD 286 and a maintenance dose of FHD 286, both administered orally. The subjects, who are not previously treated with FHD 286, commence therapy with the differentiation dose regimen initially first administered to the subject, followed by the maintenance dose regimen being administered to the subject following the completion of the differentiation dose regimen and optionally a drug holiday (i.e., a period of 1-7 days without dosing).

The differentiation dose of FHD 286, or a pharmaceutically acceptable salt thereof, is administered orally with an average daily dose of between 7.5 mg and 22.5 mg, and wherein the average daily dose during the differentiation dose regimen is at least 1.25-fold higher than the average daily dose during the maintenance dose regimen. The differentiation dose is followed by the maintenance dose of FHD 286, or a pharmaceutically acceptable salt thereof in a cyclical on/off dosing regimen.

The maintenance dose of FHD 286, or a pharmaceutically acceptable salt thereof, is administered orally with an average daily dose of between about 1.5 mg to 7.5 mg QD to the subject for a period of at least one to twenty-eight days.

The maintenance dose of FHD 286, is administered to the subject for a period of at least 1-6 months. The differentiation-maintenance cycle is repeated multiple times, as needed.

For some responsive patients, the differentiation dosing of FHD 286 is discontinued after three cycles, two cycles, or one cycle, after which the patients continue to receive the maintenance dosing of FHD 286 alone for a period of at least one month, two months, three months, four months, six months, 1 year, 2 years, 5 years, 10 years or 20 years.

Subjects receiving the differentiation dosing and maintenance dosing combination of FHD 286 can benefit from a reduction in the risk of developing differentiation syndrome and/or benefit from a reduction in the number of peripheral blasts over time, relative to a steady high-dose treatment with FHD 286 or a steady low dose treatment with FHD 286.

Example 4. Providing a subject who's pretreated with cytotoxic agent, then maintenance dosing of FHD 286 for the treatment of cancer.

Subjects with acute myeloid leukemia (AML) are treated with a maintenance dose regimen of FHD 286 administered orally. The subjects, who are previously treated with an anticancer agent to induce cell arrest and/or apoptosis in leukemic stem cells in said subject, commence treatment by first receiving a treatment with FHD 286.

The maintenance dose of FHD 286, is administered orally with an average daily dose of between about 1.5 to 7.5 mg QD to the subject for a period of at least 1-6 months.

Subjects receiving the maintenance dose regimen of FHD 286 can benefit from a reduction in the risk of developing differentiation syndrome and/or benefit from a reduction in the number of peripheral blasts over time, relative to a steady high-dose treatment with FHD 286 or a steady low dose treatment with FHD 286.

Other Embodiments

While the invention has been described in connection with specific embodiments thereof, it will be understood that invention is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

Other embodiments are in the claims.

CLAIMS

What is claimed is:

1. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject (i) a differentiation dose regimen of FHD 286, or a pharmaceutically acceptable salt thereof, and, following step (i), administering to the subject (ii) maintenance dose regimen of FHD 286, or a pharmaceutically acceptable salt thereof, wherein the differentiation dose regimen and the maintenance dose regimen together are effective to treat the cancer, and wherein the average daily dose during the differentiation dose regimen is at least 1.25-fold higher than the average daily dose during the maintenance dose regimen.

2. The method of claim 1, wherein the differentiation dose regimen (a) increases the appearance of a marker of differentiation in one or more dedifferentiated cells of the cancer, (b) decreases the appearance of a marker of dedifferentiation in one or more dedifferentiated cells of the cancer, (c) induces cell arrest in one or more dedifferentiated cells of the cancer, or (d) induces apoptosis in in one or more dedifferentiated cells of the cancer.

3. The method of claim 1 or 2, wherein, following the administration of the differentiation dose regimen, the maintenance dose regimen inhibits the proliferation of dedifferentiated cells of the cancer.

4. The method of any one of claims 1-3, wherein the differentiation dose regimen is sufficient to produce a $C_{average}$ plasma concentration level of at least 250 ng/mL in the subject, and the maintenance dose regimen is sufficient to produce a $C_{average}$ plasma concentration level of between about 80 ng/mL and about 225 ng/mL in the subject.

5. The method of any one of claims 1-4, wherein the differentiation dose regimen comprises administering daily to the subject from greater than 7.5 mg to 22.5 mg of FHD 286, or a pharmaceutically acceptable salt thereof, and the maintenance dose regimen comprises administering daily to the subject from 1.5 mg to 7.5 mg of FHD 286, or a pharmaceutically acceptable salt thereof.

6. The method of any one of claims 1-5, wherein the differentiation dose regimen comprising administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of at least one to twenty-eight days.

7. The method of claim 6, wherein the differentiation dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of at least one to fourteen days.

8. The method of claim 7, wherein the differentiation dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of at least one to ten days.

9. The method of claim 8, wherein the differentiation dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of at least one to five days.

10. The method of claim 9, wherein the differentiation dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of one day.

11. The method of any one of claims 1-5, wherein the differentiation dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject once or more every ten days to fourteen days.

12. The method of claim 11, wherein the differentiation dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject once or more weekly.

13. The method of claim 12, wherein the differentiation dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject at least once daily.

14. The method of any one of claims 1-13, wherein the differentiation dose regimen comprises administering FHD 286, or a pharmaceutically acceptable salt thereof, at an average daily dose of from greater than 7.5 mg to 22.5 mg .

15. The method of any one of claims 1-14, wherein the maintenance dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of at least one month.

16. The method of claim 15, wherein the maintenance dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of at least 3 months.

17. The method of claim 16, wherein the maintenance dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of at least six months.

18. The method of claim 17, wherein the maintenance dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of at least 12 months.

19. The method of any one of claims 1-18, wherein the maintenance dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject once or more every ten to fourteen days.

20. The method of claim 19, wherein the maintenance dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject once or more weekly.

21. The method of claim 20, wherein the maintenance dose of FHD 286 is administered to the subject at least once daily.

22. The method of any one of claims 1-21, wherein the maintenance dose regimen comprises administering FHD 286, or a pharmaceutically acceptable salt thereof, at an average daily dose of from 1.0 mg to 7.5 mg .

23. The method of any one of claims 1-22, wherein the ratio of the average daily dose of FHD 286, or a pharmaceutically acceptable salt thereof, administered during the differentiation dose regimen to the average daily dose of FHD 286, or a pharmaceutically acceptable salt thereof, administered during the maintenance dose regimen is from 10:1 to 1.25:1.

24. The method of any one of claims 1-23, wherein the final dose of the differentiation dose regimen and the first dose of the maintenance dose regimen are administered within 28 days of each other.

25. The method of any one of claims 1-24, wherein the maintenance dose regimen of FHD 286 is administered orally.

26. The method of claim 25, wherein the maintenance dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of at least one month.

27. The method of claim 26, wherein the maintenance dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of at least 3 months.

28. The method of claim 27, wherein the maintenance dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of at least six months.

29. The method of claim 28, wherein the maintenance dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject for a period of at least 12 months.

30. The method of any one of claims 1-29, wherein the maintenance dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject once or more every ten to fourteen days.

31. The method of claim 30, wherein the maintenance dose regimen comprises administration of FHD 286, or a pharmaceutically acceptable salt thereof, to the subject once or more weekly.

32. The method of claim 31, wherein the maintenance dose of FHD 286 is administered to the subject at least once daily.

33. The method of any one of claims 1-32, wherein the maintenance dose regimen of FHD 286 is administered orally.

34. The method of any one of claims 1-33, wherein the method comprises at least 21 days of treatment.

35. The method of any one of claims 1-34, wherein the method comprises at least 28 days of treatment.

36. The method of any one of claims 1-35, wherein the method comprises at least 60 days of treatment.

37. The method of any one of claims 1-36, wherein the cancer is a solid tumor.

38. The method of claim 37, wherein the solid tumor is selected from bladder, gastrointestinal tract, colon, breast, prostate, lung, kidney, liver, pancreas, ovary, head and neck, oral cavity, stomach, duodenum, small intestine, large intestine, anus, gall bladder, labium, nasopharynx, skin, uterus, male genital organ, urinary organs, skin, sarcomas, brain, or bone.

39. The method of claim 37, wherein the solid tumor is uveal melanoma, cutaneous melanoma, non-small cell and small cell lung cancers, neuroendocrine prostate cancer, or immuno-oncology (IO).

40. The method of any one of claims 1-36, wherein the cancer is a hematologic cancer.

41. The method of claim 40, wherein (a) the differentiation dose regimen is sufficient to induce cell arrest and/or apoptosis in leukemic stem cells in a subject and (b) the maintenance dose regimen is insufficient to induce cell arrest and/or apoptosis in leukemic stem cells in a subject, but sufficient to reduce the number of blast cells.

42. The method of claim 40 or 41, wherein the hematologic cancer is multiple myeloma, large cell lymphoma, acute T-cell leukemia, acute myeloid leukemia, myelodysplastic syndrome, immunoglobulin A lambda myeloma, diffuse mixed histiocytic and lymphocytic lymphoma, B-cell lymphoma, acute lymphoblastic leukemia, diffuse large cell lymphoma, or non-Hodgkin's lymphoma.

43. The method of any one of claims 40-42, wherein the hematologic cancer is acute myeloid leukemia or myelodysplastic syndrome.

44. The method of any one of claims 40-42, wherein the hematologic cancer has an inv(3) mutation, a -7/del(7q) mutation, a SF3B1 mutation, a MLLr mutation, a RUNX1 mutation, an ASXL1 mutation, a JAK2 mutation, a NRAS mutation, a KRAS mutation, a TP53 mutation, a TET2 mutation, and/or a DNMT3A mutation.

45. The method of any one of claims 1-44, wherein the method inhibits cancer cell invasion or migration in a subject having a high risk of cancer cell invasion or migration.

46. The method of any one of claims 1-44, wherein the method suppresses metastatic progression of cancer.

47. The method of any one of claims 1-44, wherein the method suppresses metastatic colonization of cancer.

48. The method of any one of claims 1-44, wherein the method slows the spread of a migrating cancer in a subject having a high risk of the spread of the migrating cancer.

49. The method of any one of claims 1-44, wherein the method reduced the rate of tumor seeding of a cancer in a subject having a high risk of tumor seeding of a cancer.

50. The method of any one of claims 1-44, wherein the method reduces or treats metastatic nodule-forming of a cancer in a subject having a high risk of metastatic nodule-forming of a cancer.

51. The method of any one of claims 1-44, wherein the method reduces metastatic risk of a cancer in a subject having a high metastatic risk.

52. The method of any one of claims 1-44, wherein the method treats metastatic cancer in a subject having a high metastatic risk.

53. The method of any one of claims 1-44, wherein the method reduces tumor heterogeneity.

FIG. 1

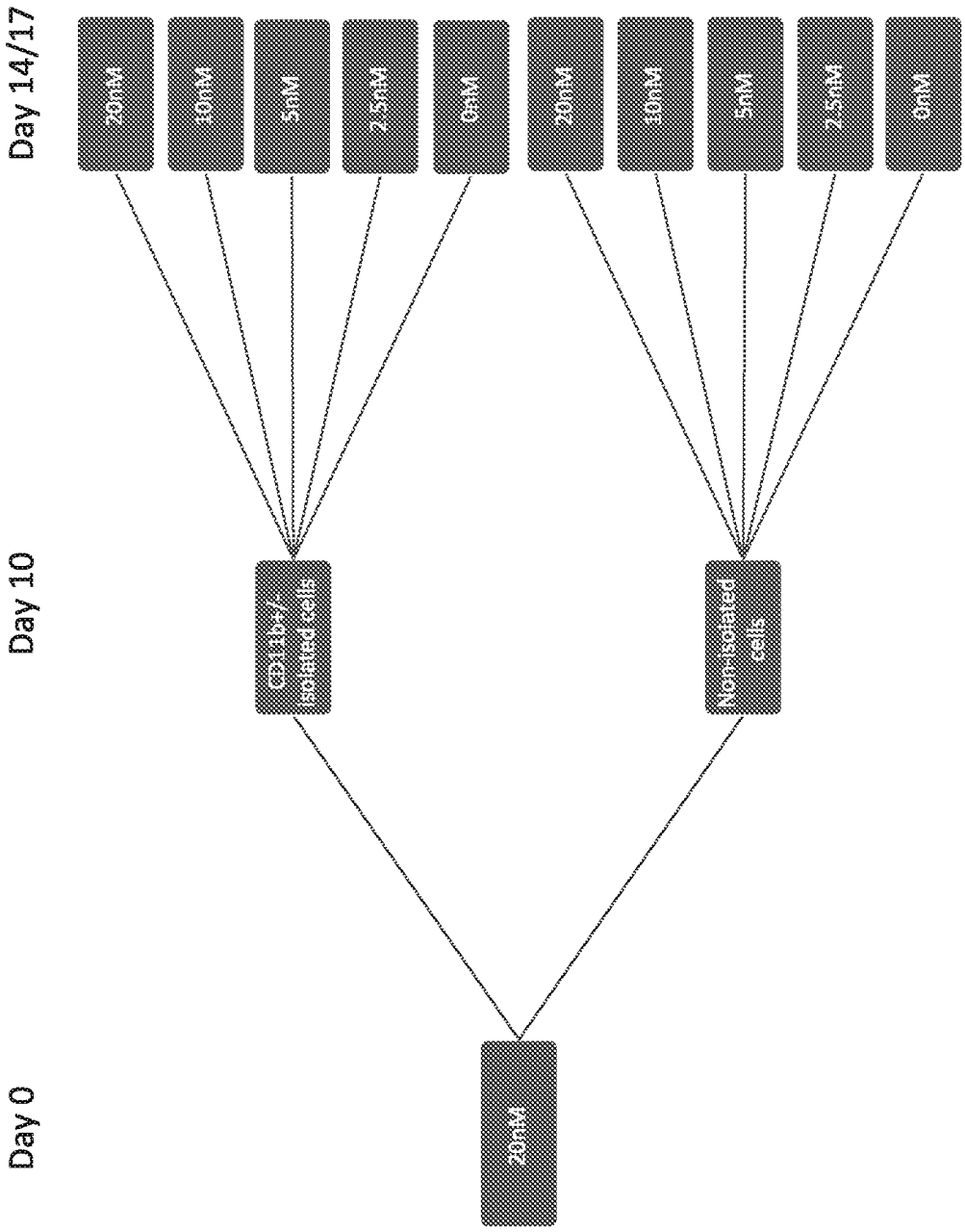

Day 14/17
20nM
10nM
5nM
2.5nM
0nM
20nM
10nM
5nM
2.5nM
0nM
Day 10
CD11b+ isolated cells
Non-isolated cells
Day 0
20nM

FIG. 2

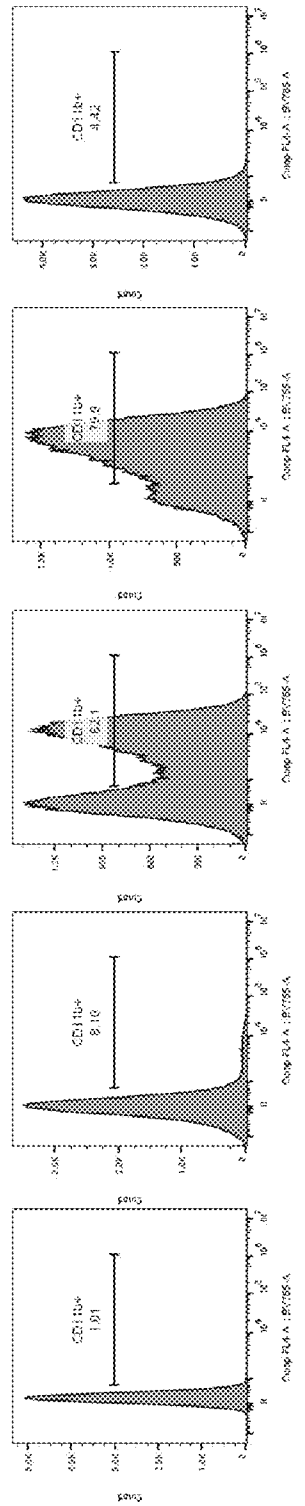

Day 10
PANEL 1:
BV785-CD11b
FITC-BRG1
APC-Ki67
Unstained
DMSO
20nM non-iso
20nM CD11b+ iso
20nM CD11b- iso
Counts
CD11b+
1.01
CD11b+
8.10
CD11b+
6.21
CD11b+
79.9
CD11b+
31.42

FIG. 3

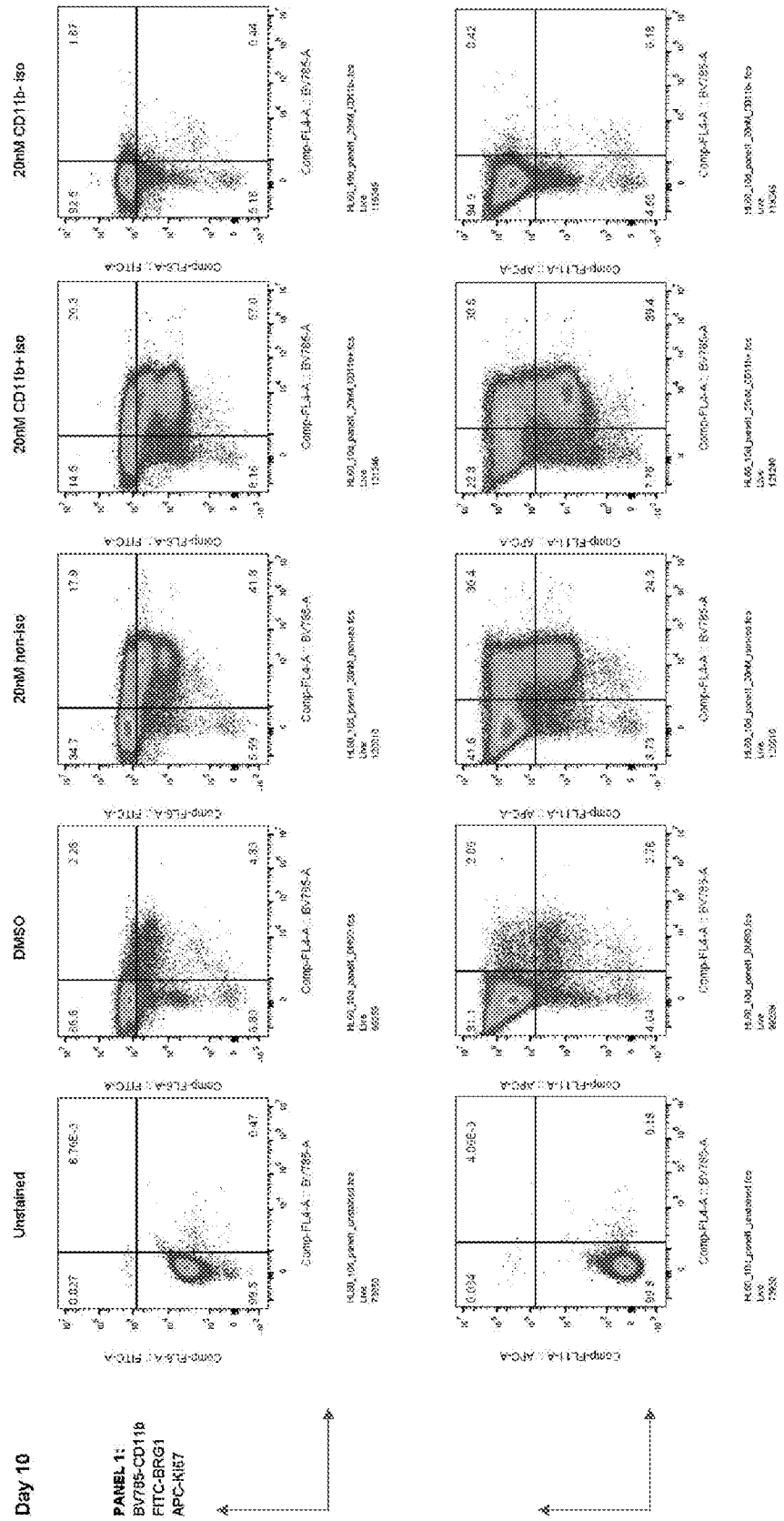

Day 10
PANEL 1:
BV785-CD11b
FITC-BRG1
APC-Ki67
Unstained
DMSO
20nM non-iso
20nM CD11b+ iso
20nM CD11b- iso

FIG. 4

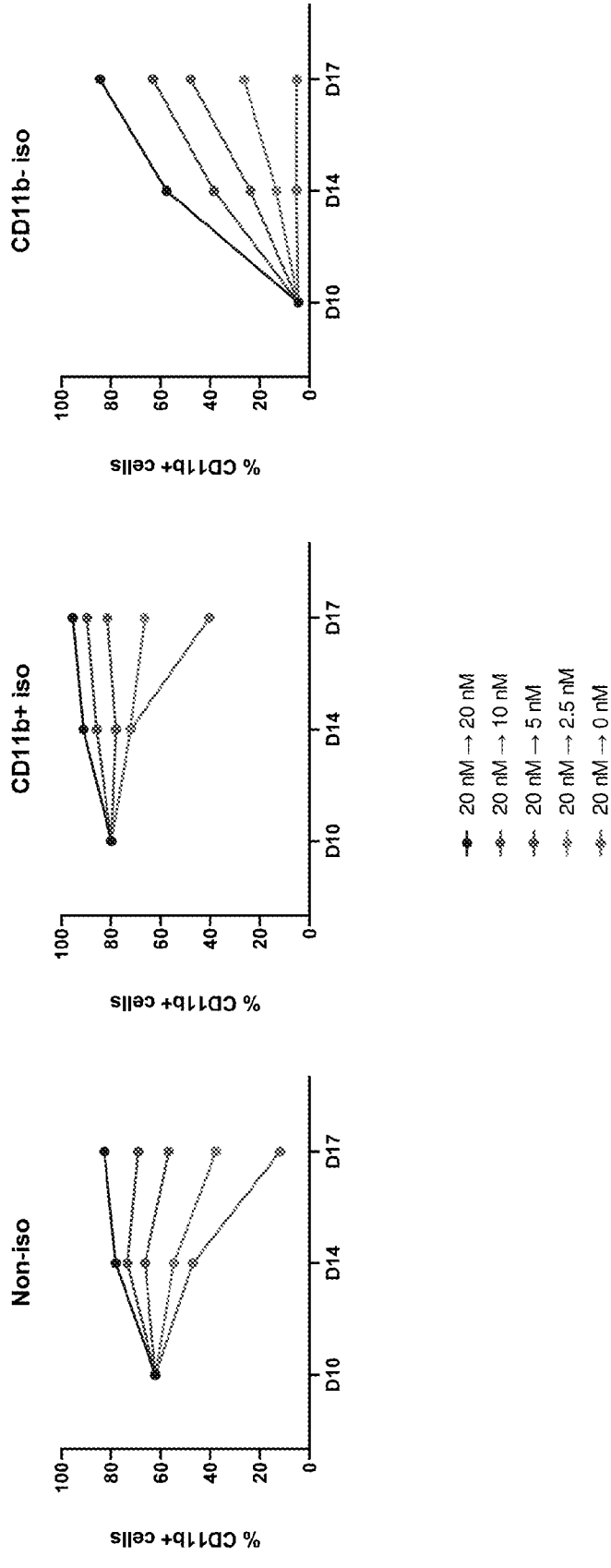
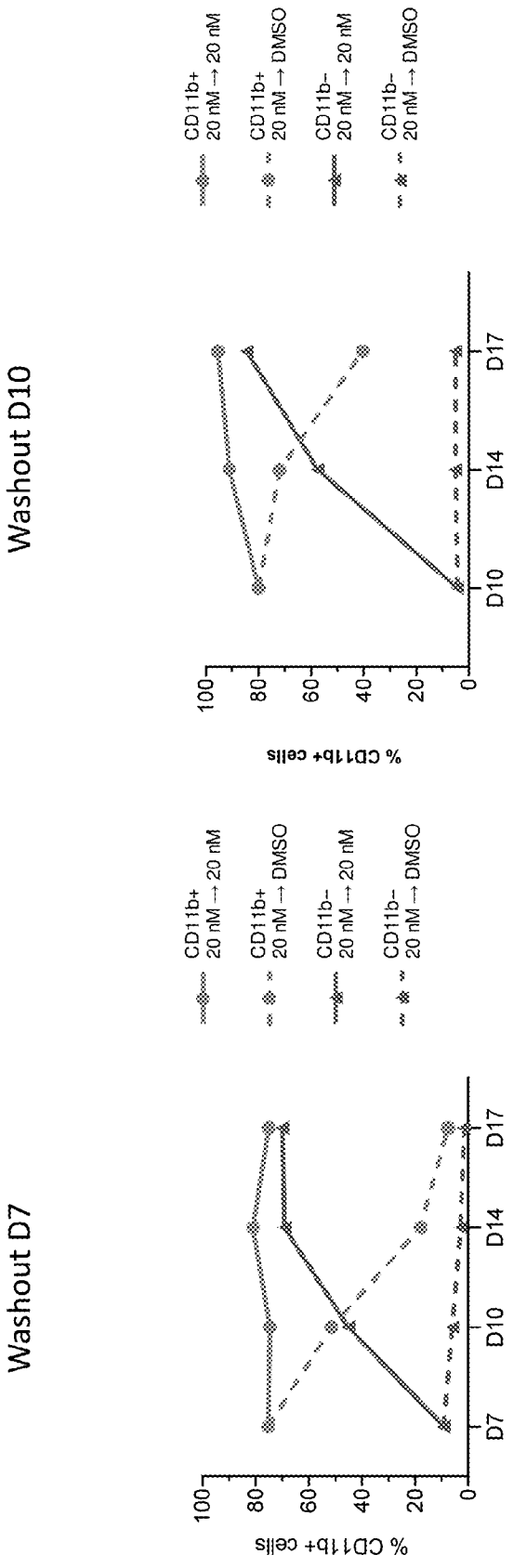

Non-iso
CD11b+ iso
CD11b- iso
% CD11b+ cells
100
80
60
40
20
0
D10
D14
D17
20 nM → 20 nM
20 nM → 10 nM
20 nM → 5 nM
20 nM → 2.5 nM
20 nM → 0 nM

FIG. 5


Washout D7
Washout D10
% CD11b+ cells
100
80
60
40
20
0
D7
D10
D14
D17
CD11b+ 20 nM → 20 nM
CD11b+ 20 nM → DMSO
CD11b− 20 nM → 20 nM
CD11b− 20 nM → DMSO

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/050660

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 31/5355*** (2024.01); ***A61P 35/00*** (2024.01); ***A61P 43/00*** (2024.01)

CPC: ***A61K 31/5355; A61P 35/00; A61P 43/00;*** *A61K 2300/00*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2021/0077486 A1 (AURIGENE DISCOVERY TECHNOLOGIES LIMITED) 18 March 2021 (18.03.2021) entire document	1-3
A	WO 2023/009834 A2 (FOGHORN THERAPEUTICS INC.) 02 February 2023 (02.02.2023) entire document	1-3
A	US 2016/0130663 A1 (NATIONAL CANCER CENTER et al.) 12 May 2016 (12.05.2016) entire document	1-3
A	COLLINS et al. "The dual BRM/BRG1 (SMARCA2/4) inhibitor FHD-286 induces differentiation in preclinical models of AML", Cancer Research; 4 April 2023; Volume 83, Issue 7 Supplement; Pgs. 1-5, [retrieved on 11.12.2024]. Retrieved from the internet: <URL: https://aacrjournals.org/cancerres/article/83/7_Supplement/2122/721920/Abstract-2122-The-dual-BRM-BRG1-SMARCA2-4>. entire document	1-3

☑ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 November 2024 (13.11.2024)	**26 November 2024 (26.11.2024)**

Name and mailing address of the ISA/US	Authorized officer
COMMISSIONER FOR PATENTS **MAIL STOP PCT, ATTN: ISA/US** **P.O. Box 1450** **Alexandria, VA 22313-1450** **UNITED STATES OF AMERICA**	**TAINA MATOS**
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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FISKUS et al. "Pre-clinical efficacy of targeting BAF complexes through inhibition of the dual ATPases BRG1 and BRM by FHD-286 in cellular models of AML", Blood; 15 November 2022; Volume 140, Issue Supplement 1; Pages 8819–8820; [retrieved on 11.12.2024]. Retrieved from the internet: <URL: https://foghorntx.com/wp-content/uploads/2023/04/Preclinical-efficacy-of-targeting-BAF-complexes-through-inhibition-of-the-dual-ATPases-BRG1-and-BRM-by-FHD286-in-cellular-models-of-AML-AACR-2023.pdf>. entire document	1-3
A	HENTEMANN, "Pharmacological profile and anti-tumor properties of FHD-286: A novel BAF inhibitor for the treatment of transcription factor-driven cancers", Cancer Research; 15 June 2022; Volume 82, Issue 12_Supplement; Abstract ND14; Pgs. 1-4, [retrieved on 11.12.2024]. Retrieved from the internet: <URL: https://aacrjournals.org/cancerres/article/82/12_Supplement/ND14/704794/Abstract-ND14-Pharmacological-profile-and-anti>. entire document	1-3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/050660

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☑ Claims Nos.: **4-53**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).